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THE PLANT DISEASE REPORTER

Issued By

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 42 **Number** 5

May 15, 1958

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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THE PLANT DISEASE REPORTER

Crops Research Division Plant Industry Station, Beltsville, Maryland
Volume 42 May 15, 1958 Number 5

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THE "PFEFFINGERKRANKHEIT" OR "ROSETTE" DISEASE IN PENNSYLVANIA SOUR CHERRY ORCHARDS[1]

F. H. Lewis

Abstract

A destructive virus disease has been found in sour cherry (Prunus cerasus) orchards in Pennsylvania. Symptoms include reduced growth, numerous rosettes of leaves and buds, death of spurs and terminals, low yields of fruit of variable maturity, and various leaf abnormalities. Some leaves were small and few in number on each spur or terminal shoot. Others were asymmetrical or narrow, sometimes with a rough surface. Some leaves bore well-developed enations. Frost bands on the fruit, necrotic areas in the fruit, and dead or severely injured Prunus mahaleb rootstocks were common and may have been due to cold injury on tissue weakened or sensitized by a virus. The symptoms suggest that this is the same disease previously described as rosette in Canada and Pfeffingen disease in Switzerland. The name rosette describes a prominent symptom on both sweet and sour cherries and seems preferable to the other names used for this disease.

In 1942, Willison and Chamberlain (8) described a disease on a single tree of sour cherry, Prunus cerasus, in Ontario, Canada, and called it rosette. While the description of the symptoms was brief, it fits the rather variable symptoms of the disease discussed in this paper.

In 1950, Blumer and Geering (3) described a destructive rosette-type disease of sweet cherries, Prunus avium, in Switzerland and called it "Pfeffingerkrankheit" after the community of Pfeffingen where the disease had been present since 1890. A similar disease was reported from the Netherlands in 1951 under the name "Eckelrader disease" (6, 7).

In 1950, Milbrath found the disease in his experimental plots in Oregon (6). The virus evidently had been brought into the plots in scions of Prunus avium, variety Esperen, originally obtained from France by the Plant Introduction Station at Chico, California. It had also been introduced in scions of Prunus cerasus var. umbraculifera obtained by the Chico station from the Arnold Arboretum. The diseased material in Oregon was destroyed by Milbrath.

Gilmer, Brase and Parker (4) in 1957 reported a disease similar to Pfeffingerkrankheit as an orchard problem in sour cherries in New York, and called it vein-clearing rosette under the assumption that it was identical with the disease briefly described and illustrated by Hildebrand (5) as rosette or vein-clearing. They stated that the disease had been present in New York for at least 15 years.

This paper reports the occurrence of rosette as a serious orchard problem in sour cherries in Pennsylvania.

SYMPTOMS OBSERVED IN ORCHARD SURVEYS

Bearing sour cherry orchards throughout the United States commonly contain a large number of trees which are carriers of the ring spot and yellows viruses (2) either with or without visible symptoms. Annual surveys over the last 15 years have given us some experience in the identification of these two diseases as they occur in this area. The symptoms observed in two orchards in 1957 were different from those previously encountered, and are believed to have been caused by the rosette virus. In certain cases, however, the rosette symptoms were associated with the symptoms of ring spot and yellows. Evidently the trees sometimes contained three or more viruses.

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[1] Authorized for publication Feb. 21, 1958, as paper No. 2236 in the Journal Series of the Pennsylvania Agricultural Experiment Station and Contribution No. 230 from the Department of Botany and Plant Pathology, Pennsylvania State University.

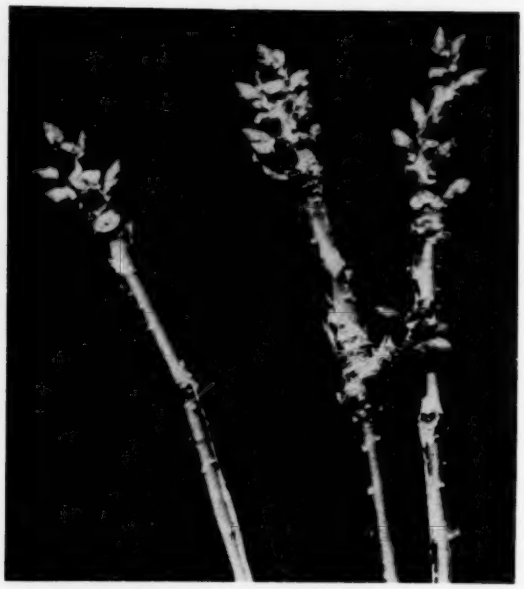

FIGURE 1. Rosette of buds on about 1/2 inch of current-season growth from rosette-diseased Montmorency sour cherry.

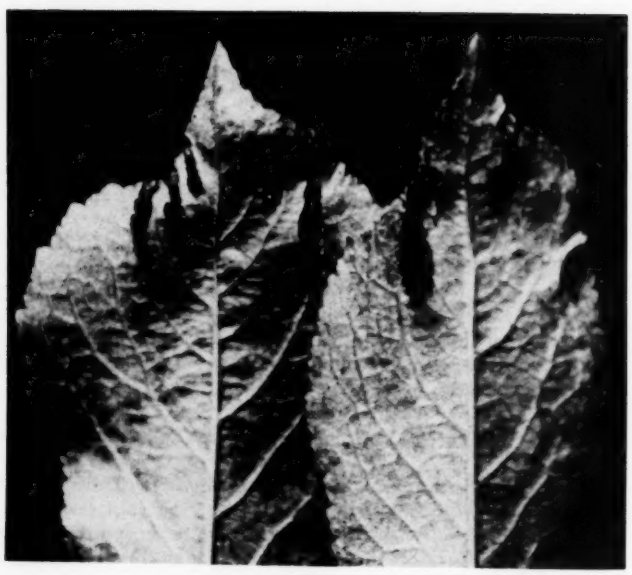

FIGURE 2. Enations on leaves from sour cherry trees with rosette.

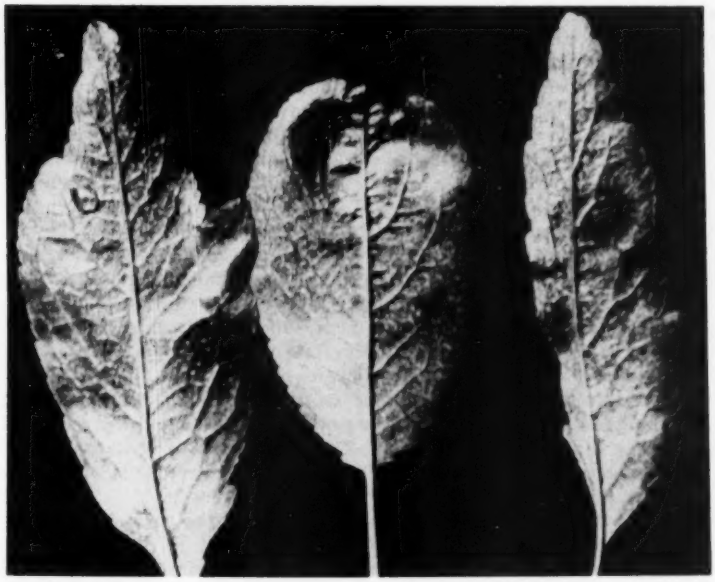

FIGURE 3. Small, malformed, asymmetrical leaves from sour cherry tree with rosette.

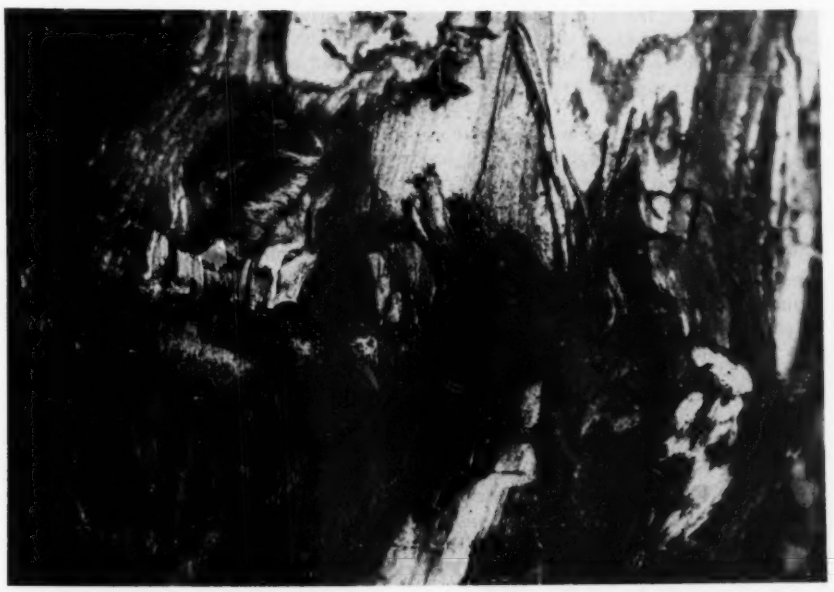

FIGURE 4. Point of scion-stock union under mature sour cherry tree showing death of Prunus mahaleb rootstock, possibly due to cold injury aggravated by rosette infection.

Death of spurs and shoots was common on rosette-diseased trees. In most cases, the necrosis consisted of dead spurs in association with the leaf and fruit symptoms described later in this paper. In a few trees, up to 50 terminal shoots per tree had died back a distance of 2 to 3 feet. This was followed by the development of vigorous lateral shoots. Since the leaves on these lateral shoots were younger than those on the remainder of the tree, an easily recognizable pattern of light-green foliage against a dark-green background resulted.

The leaves showed various abnormalities. Many of them were small and borne in clusters of two to four, with the total leaf surface no more than 25 percent of normal. Some of the leaves were narrow. Some were asymmetrical, with one side narrower than the other. The serrations were irregular. The margin of the leaf was sometimes irregular, with leaf-tip-like projections or small slit-like indentations. In asymmetrical leaves some of the lateral veins were spaced at irregular intervals. Well-developed enations were common, sometimes as various types of outgrowths or folded areas along the leaf margin but most commonly as dark-green, elongated, V-shaped outgrowths on the tip half of the lower side of the leaf. The enations usually varied from one to four per leaf on a low percentage of the leaves in that portion of the tree in an early stage of decline. Peg-like projections on the lower side of the leaf occurred either alone or with the enations.

The leaves were a dull gray-green in color on a few branches where the disease apparently had been present for a year or more. In general, however, the leaves did not show any distinct color pattern of diagnostic value. They did not fall prematurely. No distinct vein-clearing (4) was observed, although this symptom may have been present in the very early stages of growth not covered by these studies.

Terminal growth was short on many diseased trees, with a pronounced rosetting of the leaves and buds in the advanced stages of decline.

Two types of fruit symptoms were present. In one type, considered to be a symptom of rosette, there was a great variation in the size and maturity of the fruit. Some fruits were of normal size and were dark-red in color, while other fruits on the same branch were small in size and green or light-pink in color. In the second type of fruit injury, variable amounts of necrosis were present with surface lines, spots and indentations. Other fruits had russeted bands of the type normally associated with frost injury. While this fruit injury was obviously worse on trees or portions of trees with virus symptoms, we could not be certain about its relationship to the rosette disease. Identification of diseased and healthy tissue was not always possible.

The yield of fruit was very low on many trees, often less than 10 percent of normal. Even where some fruit was present the crop was not of sufficient size and uniformity to justify harvesting costs.

A few trees were observed with almost no fruit, with no leaves except for a few rosettes of misshapen leaves with a rough or corrugated surface, with some dead spurs and twigs, and with gum deposits on the trunk and scaffold branches. These trees apparently represent the most advanced stage of rosette prior to death of the trees.

In the orchards where rosette was found, some trees had died and others showed a yellow, rolled-leaf condition common with various types of root injury. It was found that the Prunus mahaleb rootstock was dead or severely injured under the trees with yellow foliage as well as under some other trees. The injury occurred at the bud union and extended downward for several inches. It usually did not extend into the lateral roots. The injury began at a point up to 12 inches below the surface of the soil. The root injury was often associated with leaf and fruit symptoms of rosette, but not in all cases.

DISCUSSION

The symptoms found in these studies are generally similar to those described from Canada, Oregon, and New York and appear to warrant the conclusion that this is the same disease as found in those areas. Observations by the writer in New York and West Virginia have led to the conclusion that the disease in those States is the same as that in Pennsylvania, although there are some differences in the symptoms observed to date. Furthermore, the diseased trees described by Adams and Kessler (1) in West Virginia were originally purchased from Pennsylvania and were grown on a farm where rosette is now present.

The symptoms on sour cherry in Pennsylvania are much like those listed by Blumer and Geering (3) as secondary or unclassified symptoms on sweet cherry in Switzerland. They list as primary symptoms slow growth, leaves of normal size with oil spots, asymmetrical form of the leaves, and sometimes curling of the leaves. These symptoms were followed in

late summer and fall by the development of mosaic and various leaf malformations. Oil spots and mosaic have not been observed on sour cherry.

The death of the mahaleb rootstocks, the necrotic areas within the fruit, and the russeted bands on the fruit may be cold injury on tissue weakened or sensitized by a virus. This idea is based on frequent association of these symptoms with recognized symptoms of virus diseases, especially rosette and ring spot.

While ringspot, yellows, and rosette of sour cherry appear to be separate diseases that may occur in combination in a single tree, studies are needed to determine symptom development following single and multiple inoculations with the causal viruses. A degree of cross-protection may occur.

If the disease found in various parts of the United States is caused by the Pfeffingen virus from Europe, how did it get here? The sour cherry trees in southern Pennsylvania are grown by local nurserymen using local budwood placed on Prunus mahaleb seedlings purchased from western nurserymen. There are few sweet cherry trees in the nurseries or near the orchards. The possibility exists that the virus was imported from Europe in mahaleb cherry seeds, and was spread into Pennsylvania in mahaleb cherry seedlings.

While the question of priority of names has not been thoroughly studied, it seems evident that a simple name which partially describes the disease has a great deal of merit. The name rosette as used by Willison and Chamberlain describes a prominent symptom on both sweet and sour cherries, and seems preferable to one of the European names.

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ROSETTE OR "PFEFFINGERKRANKHEIT" ON MONTMORENCY SOUR CHERRY IN WEST VIRGINIA[1]

Robert E. Adams and Kenneth J. Kessler, Jr.[2]

Abstract

A serious disease has been found affecting Montmorency sour cherry trees in West Virginia. On the basis of symptoms and the successful graft transmission of the disease there is little doubt that this is the Pfeffingerkrankheit, or Eckelrader disease known in Europe. Symptoms of the disease in West Virginia are described in detail and information on rate of natural spread is presented.

This disease, termed Rosette in this paper, is considered to be the same as Pfeffingerkrankheit (1, 5, 7, 10, 11, 12), Eckelrader disease (7, 8, 9), Rosette (13), Rosette or Vein Clearing (3), and Vein Clearing Rosette (2). The name Rosette first used by Willison and Chamberlain (13) and later by Hildebrand (3) is considered preferable to the other names. It is descriptive of a major symptom of the disease that appears to be constant where the disease is known, from the Niagara Peninsula, Ontario, Canada to West Virginia, and in Holland, Germany, and Switzerland. During the growing season of 1956, rosette was found in two Montmorency sour cherry orchards in West Virginia. Transmission studies and a survey of the most severely affected orchard were initiated immediately. The purpose of this paper is to describe and illustrate this disease in West Virginia, to report on natural spread, and to present evidence of graft transmission.

SYMPTOMS

The most striking symptom of this disease in West Virginia is dieback (Fig. 1). This symptom when present may vary in extent from one affected terminal to complete death of the tree. It is common to find one-quarter to one-half of the tree dead, with the remainder of the tree normal in appearance except for a transition zone of malformed leaves and rosetted terminal growth arising from between the necrotic and healthy portions of the tree. Frequently many vigorously growing shoots are produced immediately below the killed portions of the tree. Leaves on these shoots may be dwarfed and malformed but in some cases they were found to be normal in appearance.

Rosetting of terminal growth is especially pronounced and in many instances extremely severe (Fig. 2, 3, 4). Gradients of symptom severity have been observed to range from one affected (rosetted) terminal to involvement of the entire tree. Branches may show symptoms only on portions distal to the main stem. Diseased portions appear in sharp contrast to normal portions and the dividing line between diseased and normal is narrow.

Symptomatic foliage shows various abnormalities. In general, diseased leaves are smaller than normal, cupped, and in many instances are much narrower than normal leaves (Fig. 3). Leaf margins tend to be irregular, and many are asymmetrical (Fig. 2, 3, 5). Also, many leaves are truncate (Fig. 5). Leaf serrations show a pronounced irregularity. Both upper and lower surfaces of diseased leaves are rough (Fig. 2, 3, 5). This roughening appears to be the result of an overgrowth or irregularity of the veins. The overgrowths along the

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[1]Published with the approval of the director of the West Virginia Agricultural Experiment Station as scientific paper No. 576. Portions of this paper are based on a thesis presented by the junior author to the Graduate School of West Virginia University in partial fulfillment of the requirements for the Master of Science degree (5). This work has been conducted on a project contributing to Regional Project NE-14. The constructive criticisms, free exchange of ideas, and in some instances active participation in the work by individuals working on similar projects in Pennsylvania and New York is hereby acknowledged.

[2]Assistant Plant Pathologist and Graduate Assistant in Plant Pathology respectively, Department of Plant Pathology, Bacteriology, and Entomology, West Virginia University, Morgantown, West Virginia.

FIGURE 1. Montmorency sour cherry tree showing severe dieback associated with rosette in West Virginia.

FIGURE 2. Rosetted terminal of Montmorency sour cherry affected with rosette, showing narrow leaves, asymmetrical leaves, roughening of leaf surfaces, irregular serrations, and blossom blast.

FIGURE 3. Close-up view of Montmorency terminal with rosette, showing asymmetrical leaves, irregular leaf serrations, irregular leaf margins, roughened foliage surfaces, and narrow leaves.

FIGURE 4. Montmorency terminals showing extreme rosetting, dwarfed leaves, blossom blast, and bud abscission.

FIGURE 5. Leaves of Montmorency sour cherry tree with rosette, showing truncate leaves, asymmetrical leaves, and roughened leaf surfaces.

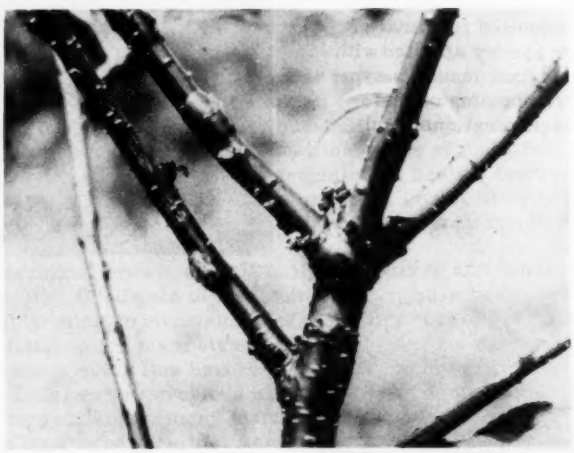

FIGURE 6. Close-up view of a branch of Montmorency sour cherry tree with rosette, showing gum pockets within the bark.

the larger veins on the lower surfaces of the leaves appeared as if they might give rise to enations. However, repeated observations over a period of 2 years have failed to reveal any true enations on any of the diseased trees. Foliage produced on diseased trees or diseased portions of trees is abnormally light green during the early part of the growing season. This symptom is easily seen from a distance and readily serves to identify diseased trees. Later in the growing season, this color difference is not great and is difficult to determine.

In 1956, blossoms on diseased branches were produced on very short pedicels. They blasted at the balloon stage. Shortly after this the buds from which the blasted blossoms arose abscised (Fig. 4). There were no blossoms on diseased portions of trees in 1957. We have found no fruit on diseased portions of trees.

Gum pockets were found in the bark of most diseased trees (Fig. 6). This symptom was not present on all trees but was encountered with great frequency and should be considered a part of the symptom complex.

Root symptoms were similar to or identical with those found in Pennsylvania[3].

All the symptoms, here described, are most pronounced during spring and early summer.

TRANSMISSION TRIALS

Several Elberta peach seedlings were inoculated in August, 1956, with diseased budwood, approximately 2 inches above ground level. By the spring of 1957, all of the inoculated seedlings were dead above the point of inoculation. The first few leaves on shoots produced below the point of inoculation were mottled. Subsequent foliage was smaller than normal but was otherwise symptomless.

Virus-free Montmorency budlings growing in pots in the greenhouse were inoculated with rosette-diseased budwood, and after 1 month placed in a cold room for a dormant treatment at 34° to 37° F. After 4 months in storage, the trees were removed to grow under greenhouse conditions. Gum pockets were produced on the branches of all inoculated trees, but their foliage appeared normal. Inoculated trees died shortly after growth started while control trees continued to grow normally. This suggests that gum production may not be due to low temperature injury, unless diseased tissue is sensitive to cold, as suggested by Lewis (6).

In 1956, three mature Montmorency trees growing at the Kearneysville station, known to be infected by necrotic ringspot and yellows, were inoculated with rosette-diseased budwood. In 1957, one inoculated branch produced abnormal, light green foliage typical of rosette. No fruit was produced on this branch.

During July 1957, three rosette-affected orchard trees were selected for further study. One Mazzard seedling, one Elberta seedling, and one Montmorency budling growing in pots were approach-grafted to each of the diseased orchard trees. Within 3 weeks, all of the foliage on the three Mazzard seedlings was dead. The seedling wood was obviously alive, and union between the seedlings and the orchard trees had been made. The foliage on the peach seedlings and the Montmorency budlings was clearly abnormal. None of these trees made growth following sufficient cold treatment to break dormancy. The reason for this behavior is not known but it is suspected that a massive dose of virus inoculum received because of the method of transfer used was responsible.

RESULTS OF SURVEY IN ONE ORCHARD

The orchard selected for survey contained 2871 trees. In 1956, when the survey was initiated, the trees were 8 years old, and most were making unusually vigorous growth. Symptoms of rosette were found on 29 percent (844) of the trees. Shock symptoms of necrotic ringspot were found on 3 percent of the trees, and sour cherry yellows was found on 1 percent. In 1957, 177 additional trees showed rosette symptoms and 147 trees that showed symptoms in 1956 were dead in 1957. This provides evidence that the disease spreads rapidly and indicates that an efficient vector is present. Several trees that showed rosette in 1956 showed shock symptoms of necrotic ringspot in 1957 as well as the rosette symptoms. This suggests that rosette may spread independently of ringspot. It is also in agreement with the findings in Pennsylvania (6) and with indexing of West Virginia material in Pennsylvania by F. H. Lewis, which show that rosette is not always associated with ringspot.

The variation in symptoms reported in New York (2, 3), Canada (13), Pennsylvania (6),

[3]See paper by F. H. Lewis, here published concurrently (6). Dr. Lewis has actively participated in some phases of this work.

and West Virginia is the result mainly of minor differences in severity of symptom expression which are considered to be due to the influence of different climatic conditions on host response.

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FIELD SYMPTOMS INDICATE OCCURRENCE OF "ALFALFA DWARF" OR "PIERCE'S DISEASE" VIRUS IN RHODE ISLAND[1]

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Summary

In 1956 and 1957 abnormalities were observed in alfalfa and grape in Rhode Island which closely resembled the symptoms induced by infections of the virus Morsus suffodiens Holmes. The ecology of these abnormalities, including considerations of potential insect vectors, is compared with that known for dwarf of alfalfa and Pierce's disease of grape. Marked similarities in this comparison indicate that M. suffodiens may be present in Rhode Island, and that a widely distributed leafhopper, Draeculacephala antica (Walker) may be an insect vector, should the virus be proven to occur in the State.

INTRODUCTION

Alfalfa, a basically important forage crop in Rhode Island, has been grown in the State for many years with varying degrees of success. One of the principal problems with the crop in Rhode Island, and the Northeast, is that it is short-lived in this region, as compared with many other alfalfa-producing areas. This problem has locally been called a lack of "persistency".

Investigators at the Rhode Island Agricultural Experiment Station have been aware of this problem and have attacked it from many seemingly proper and logical points. Soils have been thoroughly classified (27, 28, 29)[3], and some major element nutritional experiments have been completed (20, 26). Variety trials, culture investigations, and management studies have been conducted for many years and are continuing. A breeding program has produced Narragansett, a variety adapted to the Northeast (21). Diseases of alfalfa in the Northeast have been studied (17) and, while there is no comprehensive work known to the author devoted solely to the insect pests of alfalfa in this region, many individual insect species found commonly on alfalfa, such as those mentioned by Wheeler (35), do occur. None of these pests, however, has been implicated in the problem of persistency. Climatic factors have been considered as possible causes of "winter killing" especially from the aspect of freezing and frost heaving. It is possible that these factors, coupled with widely fluctuating winter temperatures, could be responsible for at least part of the persistency problem. If this were the case, however, direct observation and correlation with weather data should show a relationship which thus cannot be demonstrated.

After a careful consideration of the investigations to date, no single factor or combination of factors can be pointed to as being directly responsible for the lack of persistency of alfalfa in Rhode Island. At present the average economic life of a good plantation of alfalfa is 4 years or less[4].

In 1954 the possibility of virus infection in the crop was considered and symptoms similar to those of alfalfa "dwarf" caused by infections of the virus, Morsus suffodiens Holmes, listed by Holmes (14) were noted[5]. The author began field investigations of this problem late in September 1956 soon after joining the staff of the Station. Symptoms observed in experimental and commercial alfalfa plantings and in an experimental vineyard in the autumn of 1956 were strongly suggestive that both alfalfa and grapes were infected with M. suffodiens. The field ecology of the problem was studied and a particular search was made for possible vectors throughout the growing season of 1957.

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[3]Numbers in parentheses refer to "Literature Cited".

[4]Longevity estimate of alfalfa given the author by James E. Sheehan, Assistant Agronomist, Rhode Island Agricultural Experiment Station.

[5]Interdepartmental memorandum of F. L. Howard, Rhode Island Agricultural Experiment Station.

FIELD SYMPTOMS ON ALFALFA

Symptoms similar to those induced by infections of M. suffodiens were noted in alfalfa in practically all fields observed. The syndrome of the symptoms in affected plants taken in the seasonal cycle was as follows: 1) Early in the spring certain plants were slow to start and produced weak growth. 2) As the season progressed these plants grew more slowly than normal-appearing ones and by the time of first cutting presented a "witches' broom" appearance. This was due to a proliferation of the buds in the leaf axils on the stems and to a development of an abnormally large number of thin, weak stems from the crown (see Figure 1). The size of individual leaves was greatly reduced and the shape altered to what is locally called a "mouse ear" condition (see Figure 2). 3) At the time of first cutting, and at succeeding cuttings, the height of the affected plants ranged from one-eighth normal to almost normal. 4) From mid- to late-season it was observed that affected plants failed to set seed, whereas normal appearing plants set seed profusely. 5) Throughout the season the coloration of the "dwarfed" plants was greyish-green compared with the bright green of normal plants.

During the summer of 1957 plants showing the above abnormalities were dug and the roots were examined by peeling and then splitting. In more than 80 percent of 200 plants the medulla of the roots showed a yellowish to tan discoloration similar to that which occurs in alfalfa plants known to be infected with M. suffodiens. Microscopic examination of a group of these roots failed to disclose the presence of a visible organism.

In several 2- to 3-year-old plantings the occurrence of affected plants could be associated with grassy borders of the fields or with low spots where grasses had succeeded alfalfa soon after planting. These low spots were often boggy and the grasses in them remained green throughout the season. Because of this they proved to be good locations for vector collection. In older fields dwarfed plants could be found scattered throughout the plantings as well as in concentrations along the borders. The pattern of the occurrence of these plants conformed to that of a persistent type virus disease spread by an insect vector, and with known spread patterns for alfalfa dwarf.

It is common practice in Rhode Island to plant general purpose mixtures containing varying percentages of alfalfa, Medicago sativa L., brome grasses, Bromus inermis Leyss. and Bromus arvensis L., red clover, Trifolium pratense L., alsike clover, Trifolium hybridum L., ladino clover, Trifolium repens L. var. latum McCarthy, and timothy, Phleum pratense L. (33, 34). These species, with the exception of the brome grasses, are all reported by Freitag (9) in the host range of M. suffodiens. In fields 3 to 4 years of age planted to these mixtures scattered alfalfa plants showed varying degrees of the described symptoms.

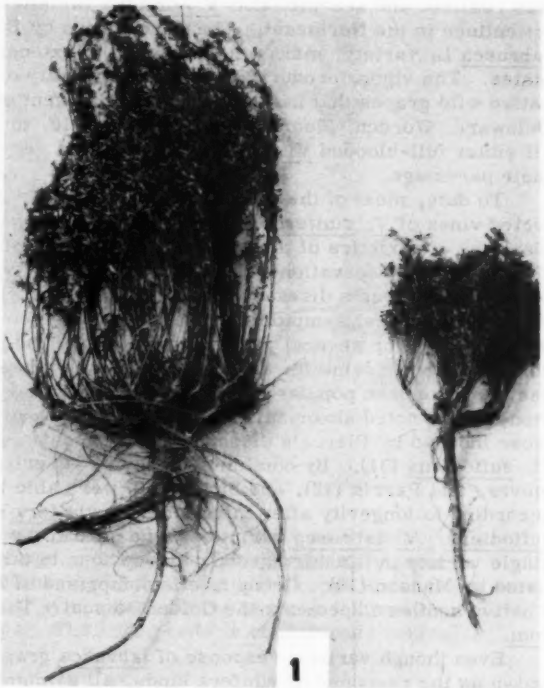
In fields 6 to 12 years old, that were originally pure stands of alfalfa, only widely scattered individual plants remained and these were dwarfed in practically all cases. Two fields planted to alfalfa in the fall of 1956 were closely observed during 1957. There were some broadleaved weeds and grasses in these fields, but in general the alfalfa stand was good and not a single plant was observed showing abnormal growth.

FIELD SYMPTOMS ON GRAPE

Observations of both wild and cultivated grapes were made concurrently with the above studies of alfalfa, since M. suffodiens infections in grape incite Pierce's disease (13). Unfortunately there were no European grapes, Vitis vinifera L., available for observation since varieties of this species are not grown in Rhode Island. The reasons for this are given in detail by Hedrick (11) and Bailey (1) in portions of their works dealing with the history of attempts to culture V. vinifera varieties in the Northeastern United States and southward along the Atlantic Seaboard. From the time of discovery of the continent by the Norsemen, who named the area Vineland because of the profusion of indigenous wild grapes they found, through the period of colonization, it was believed the "vine", V. vinifera, should grow well here. Many well-supported, long range, exhaustive attempts by expert viticulturists to grow V. vinifera varieties imported from Europe were conducted over a period of about 200 years. All of these attempts ultimately resulted in failure. Even though the vines grew well in the beginning they soon lost "vigor", became "sickly", "degenerate" or "exhausted" and died[6]. While the descriptions are by no means complete, there are notable particular symptoms given by Dufour (6), Coleman (4), Reddick (24, 25), and others as indicated by Bailey (1),

[6]Similar descriptive terms such as "sickly", "degeneration", "running out" etc. were used in Florida to describe an abnormal condition in grapes now proven to be Pierce's disease.

FIGURE 1. "Healthy" and "suspect" field-collected alfalfa plants. Plant on left is considered normal; over-all length 3 feet. Note robust stems, broad leaves, open crown and normal flowering. Plant on right exhibits symptoms similar to those produced by M. suffodiens in alfalfa. Over-all length 1 1/2 feet. Note comparatively large number of thin stunted stems, small narrow leaves, dense crown and absence of flowers.



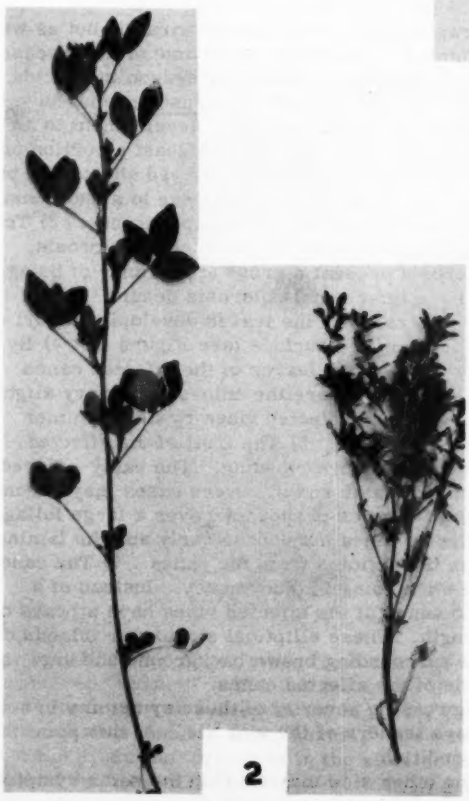


FIGURE 2. Close-up of individual stems taken from respective plants shown in Figure 1. Note the marked stunting, the proliferation of shoots from the axillary buds, and the change in shape and smaller size of the leaflets of the stem from the "suspect" plant. Stem on the left from the plant considered "healthy".

which taken collectively strongly resemble those of Pierce's disease. By the mid 1800's it was realized and accepted that V. vinifera varieties would not do for the establishment of viticulture in the Northeast. The introduction by Bull in 1853 of the Concord grape, a Vitis labrusca L. variety, marks the beginning of extensive grape culture in the Eastern United States. The vigor, productivity, and adaptability of the Concord stimulated the breeding of native wild grapes that has led to the development of such well known varieties as Niagara, Delaware, Worden, Moore's Early, and Lucile, to mention only a few. These varieties are all either full-blooded Vitis labrusca or have a very high percentage of labrusca blood in their parentage.

To date, most of the descriptions of Pierce's disease of grape have been made from infected vines of V. vinifera (12, 23) in California, where principal production is based on plantings of varieties of this species. It is interesting to note, however, that Hewitt et al. (12) did make observations on vines of Catawba, Iona, Pierce, and Concord known to be infected with Pierce's disease virus, and these varieties are included in their list of grapes showing nontypical symptoms of the disease. According to Hewitt et al. (12) Catawba and Iona may live for several years after infection and produce good crops, and Pierce and Concord may do the same for as long as 5 years. These are all V. labrusca varieties in current use, or have been popular grape varieties in the past. Stoner, Stover, and Parris (32), and Stoner (30), noted abnormalities in labrusca varieties in Florida which were compared with those induced by Pierce's disease. These plants were subsequently shown to be infected with M. suffodiens (31). By combining personal experiences and a review of the literature Stoner, Stover, and Parris (32), and Stoner (30) were able to develop a graded series of grape classes according to longevity after infection. V. vinifera varieties were most susceptible to M. suffodiens. V. labrusca varieties were next in the scale, followed closely by Catawba as a single variety in the third group. Group four in this series contains Texas Post Oak Hybrids listed by Munson (19). Group five is comprised of Florida hybrids of Vitis simpsoni Munson, a native southern species x the Golden Muscat. The sixth group was wild plants of V. simpsoni.

Even though varietal response of labrusca grapes to Pierce's disease virus is not as well known as the response of vinifera kinds, all symptoms of the known syndrome of the disease in vinifera varieties do occur in labrusca grapes at some time during the season in Florida. Similar symptoms have now been observed in both wild and cultivated labrusca grapes in Rhode Island during 1957 and the chronological order of typical symptom development is as follows: 1) In affected plants there is a delay of growth in the spring on at least a portion or on one side of the plant. 2) After a lapse of 4 to 6 weeks of growth the delayed shoots can be seen to be less vigorous than normal ones and they are somewhat shorter due to a shortening of the internodes (see Figure 3). This gives the plants an asymmetrical appearance. 3) The basal leaves of the affected canes at this time may begin to show an interveinal chlorosis, depending on the variety. Those showing the chlorosis present a gross appearance of being lighter than normal in coloration. In varieties where interveinal chlorosis occurs the condition becomes more pronounced as the season advances, with the leaves developing a coriaceous texture and a decidedly glossy appearance of the upper surface (see Figure 4). 4) By mid-July there may be a severe marginal burning of the basal leaves of the affected canes previously showing interveinal chlorosis. In the varieties where the chlorosis is very slight or absent the marginal burning may be the only symptom. Affected vines by mid-summer may have a general aspect of unseasonal autumnal coloration. 5) The fruit of the affected vines may color early thus giving the impression of premature ripening. The early-colored berries, however, may be somewhat flat and insipid to taste and in severe cases may become flaccid and withered, if the marginal burning of the leaves is pronounced over a large foliage area (see Figure 3). 6) Fall defoliation of the affected vines may occur early and the laminae of the leaves abscise from the petioles rather than the petioles from the canes. 7) The canes of the affected vines may not always ripen evenly when going into dormancy. Instead of a light, bright brown to brownish-red coloration the canes of the affected vines have streaks of green, relatively succulent tissues along their length. These elliptical streaks or islands of immature tissue may be slightly raised above the surrounding brown background and are, in some varieties, confined to the internodal sections of the affected canes.

Vines that are profoundly affected show an increasing severity of these symptoms in succeeding seasons until after a few years one or more leaders of the vine die. At this point the term "dead arm" has been used to describe the condition.

After the death of one side of a vine leader the other side may develop the same symptoms. It will follow the same general decline toward death. Adventitious buds may sprout below the

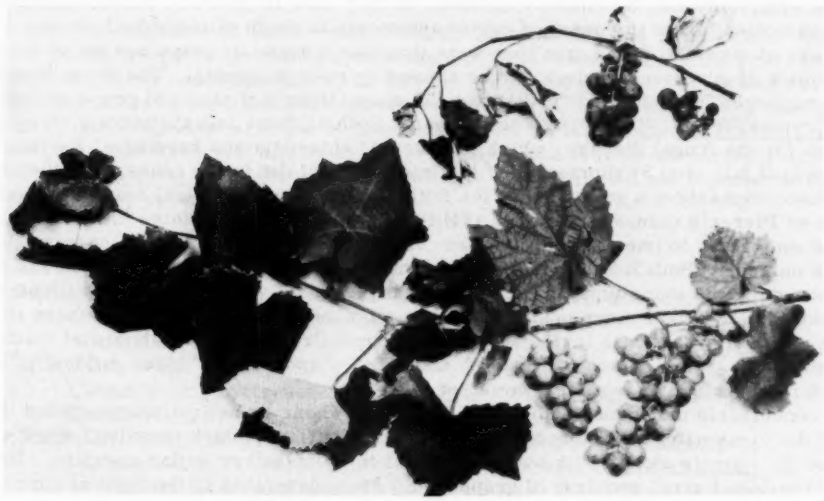


FIGURE 3. Whole canes collected from field-grown vines of Vitis labrusca. Note the scorched appearance of foliage, stunting (due to shortening of internodes), premature coloration and withering of the fruit in the upper shoot taken from an affected vine. These are all symptoms similar to those induced by Morsus suffodiens infection in grape. The lower cane is considered "normal".

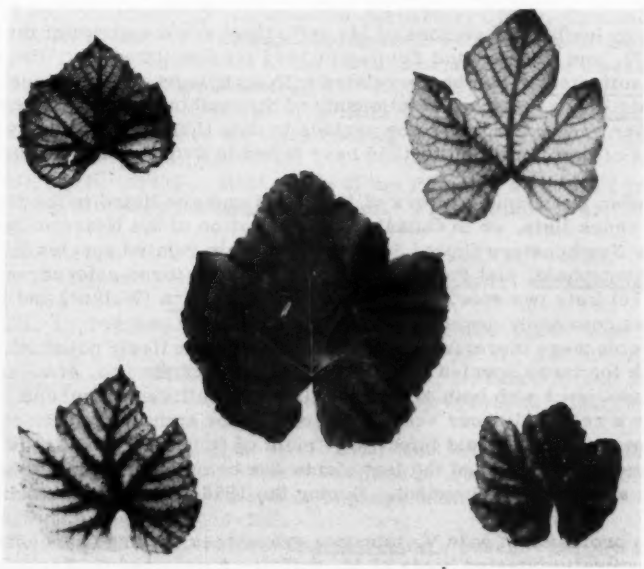


FIGURE 4. Leaves taken from field-grown Vitis labrusca grapevines. Center leaf is considered "normal". The two leaves on the left exhibit a clearly defined interveinal chlorosis while in the two leaves on the right the chlorosis is more diffuse. All four chlorotic leaves are somewhat smaller than "normal" and were taken from the basal portion of affected shoots. Note the slight malformation in the affected leaves. Such abnormalities are similar to those induced by Morsus suffodiens infections in grape.

dead portion of the vine, or at ground level, but the shoots developing from these buds will soon show symptoms and the entire vine dies. It may take 10 years or more, with some labrusca varieties, from the onset of initial symptoms to death of individual vines.

The use of the term "dead arm" for more than one disease of grape has led to some confusion. Death of grapevine leaders can be caused by several agents. The term "dead arm" has been employed by both Reddick (25) and Coleman (4) for a disease of grape caused by the fungus, Cryptosporella viticola (Reddick) Shear. Both of these investigators give symptom syndromes for the fungal disease, which include: 1) chlorosis and burning of the foliage, 2) premature leaf fall, and 3) shortening of the internodes of the grape canes. Reddick (24) in addition also emphasises a withering of the fruit. These are all typical symptoms in the syndrome of Pierce's disease. Reddick (24) in a list of symptoms states "Any of the above conditions may refer to one arm only and any vine may show only a few[7] to nearly all of the conditions named." Both Reddick (24) and Coleman (4) stress leaf malformation and size reduction as significant symptoms of the disease caused by C. viticola. Certain illustrations in their publications show vines bearing foliage with abnormalities similar to those described by Hewitt et al. (12) as "little leaf" caused by a zinc deficiency and an abnormal condition known under the varying names of "Spanish measles", "apoplexy", "black mildew", "esca", and "black measles" on V. vinifera varieties.

It is conceivable that both Reddick and Coleman, whose investigations preceded the discovery of the virus nature of Pierce's disease, little leaf, and black measles, were working with either C. viticola alone, with one of these other troubles, or with a complex. Re-examination of the "dead arm" problem of grape would seem desirable in the light of current knowledge.

The syndrome of Pierce's disease in V. vinifera varieties is very similar to the above symptoms observed in V. labrusca varieties in Rhode Island, with the exception that there is generally a greater intensity and in most cases a more rapid progress in death of V. vinifera vines. In some cases individual plants of V. vinifera varieties have been known to collapse and die from the disease in a single season after the onset of discernible symptoms (23).

OBSERVATIONS OF POTENTIAL INSECT VECTORS

All of the proven leafhopper vectors of M. suffodiens are members of the Tettigellinae. Frazier in 1944 (7), and Frazier and Freitag in 1946 (8) postulated that "the ability to transmit the virus (M. suffodiens)[8] can be correlated with a phylogenetic relationship of the vectors within the Tettigoniellinae." Such a relationship of the leafhopper vectors can be seen in all of the new leafhopper species proven to be vectors to date (10, 31). All vector tests made with leafhoppers outside of this family up to 1954 have failed to demonstrate transmission of the virus (5).

None of the known leafhopper vectors of M. suffodiens are listed in the New York (18) or Connecticut (2, 3) check lists, or in Oman's "Classification of the Nearctic leafhoppers" (22), as occurring in the Northeastern United States, but closely related species in the genera Helochara, Draeculacephala, and Pagaronia are listed in all three references. In Rhode Island, Kerr (15, 16) lists two species, Draeculacephala antica (Walker) and Helochara communis Fitch, as commonly occurring on lawns and forage crops.

These two species were therefore considered as the most likely potential vectors in Rhode Island. The search for these species in 1957 revealed that of the two, Draeculacephala antica was consistently associated with both grape and alfalfa in sufficient numbers, even should the species prove to be a relatively poor vector, to account for both the pattern of occurrence and the number of affected plants. Field infectivity trials of D. antica were made to healthy alfalfa seedlings in the summer of 1957 and the test plants are being held in the greenhouse over the winter for possible symptom development. During the 1958 season the search for other vectors will continue.

In addition to a profusion of wild V. labrusca grapevines, a large percentage of the known experimental and naturally infected hosts of M. suffodiens reported by Freitag (9) occur and are widely distributed in Rhode Island. Most of the species listed by Stuckey (33, 34) for use in Rhode Island in pastures and as forage crops are in the known host range of the virus. Some of these plants, especially the clovers, exhibit symptoms in the field similar to those induced by M. suffodiens infections.

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[7]Underscoring by the author.

[8]Parenthetical insertion by the author.

DISCUSSION

The striking similarities of the "dwarf" condition of alfalfa and of so-called "dead arm" of grape in Rhode Island to the known symptoms and field ecology of alfalfa dwarf and Pierce's disease in these hosts caused by infection with the virus Morsus suffodiens lead to the hypotheses that this virus occurs in Rhode Island, and that Draeculacephala antica may be a vector of the virus. Investigations are now in progress to test these hypotheses.

The lack of persistence of alfalfa in Rhode Island could at least be partially attributed to infections of M. suffodiens, if the virus is proven to occur in the State together with an appropriate insect vector or vectors.

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TRANSMISSION BY MYZUS PERSICAE (SULZ.) OF THE INTERNAL CORK VIRUS OF SWEETPOTATOES[1]

H. W. Rankin and J. H. Girardeau, Jr.[2]

When Nusbaum (4) first described internal cork of sweetpotatoes he noted both root and leaf symptoms. His report was based on experiments in which he used halved cork-free potatoes. In one half of each potato he core-grafted plugs from corky Porto Ricos. The potato halves with the corky plugs produced sprouts with definite leaf symptoms while the ungrafted halves produced sprouts free of symptoms. He states, however, that late season transmission of the internal cork virus under field conditions often failed to produce leaf symptoms. It is recognized by some workers that leaf symptoms are not always a reliable internal cork symptom due, perhaps, to environmental conditions, age of the plants, and the great interchange of breeding material throughout the country in the past 15 years. Doolittle and Harter (1) have called attention to the fact that several virus diseases of sweetpotato are of fairly common occurrence. Martin (3) suggests that more than one virus may be involved in sweetpotato internal cork leaf symptoms as now noted.

Nusbaum was unable to transmit internal cork mechanically but did not obtain transmission by grafting. He conducted insect transmission studies using the leaf-hopper, Empoasca fabae (Harr.), and aphid Myzus persicae (Sulz.). The leaf-hopper tests gave negative results. When aphids were used, indistinct mottling and vein clearing of the young leaves were noted but the succession of characteristic symptoms failed to develop. He considered the ability of Myzus persicae to transmit internal cork still in doubt. Rankin (5), using root symptoms, reported aphid transmission of the virus in Porto Rico sweetpotatoes but discovered he had used a mixed population of Myzus persicae and Macrosiphum gei Koch. Hildebrand (2), using Myzus persicae as vector and leaf symptoms as indicators, reported transmission of the internal cork virus from sweetpotato to sweetpotato, sweetpotato to morning-glory, morning-glory to morning-glory, and morning-glory to sweetpotato.

Root symptoms only were used in the following report.

METHODS AND MATERIALS

A bushel of sweetpotatoes free of internal cork was obtained from Lambeth of the University of Missouri in the spring of 1957. Samples of these sweetpotatoes had been root checked in Missouri and no cork symptoms were found. After being received they were stored at 85° F for 60 days. Twenty potatoes were selected for bedding and the rest were sliced completely and examined for cork symptoms. None were found. The bedding potatoes were also sliced, leaving an unsliced piece of 1 1/2 inches of the sprout end. No cork was found in any of them. The sprout ends were then bedded in sterilized soil under a cage made of 36-mesh lumite screen. A like number of sweetpotatoes, all of which had positive internal cork symptoms, were bedded in a similar manner and also placed under a 36-mesh cage. When the sprouts had reached sufficient size, 60 draws were pulled from each cage. These were heeled-in in separate flats and placed under a third cage with foliage intermingled. A population of Myzus persicae was introduced into the cage and the aphids were allowed to feed at will for 30 days. The plants were then dusted with a 2% parathion dust. The following day the plants were removed from the cage and transplanted into ground beds in a growing house screened with 32-mesh screen. These beds had been fumigated with methyl bromide. A planting of cork-free draws was made at the same time for use as checks. Immediately after transplanting the plants were sprayed with 2 pounds of 15% parathion per 100 gallons, using a power sprayer. The house was also sprayed, inside and out, and this treatment was repeated weekly until harvest.

After the sweetpotatoes were harvested they were placed in storage at 85° F for 90 days. They were then sliced and the data recorded.

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[1]Contribution of the Department of Plant Pathology, Coastal Plain Experiment Station, Tifton, Georgia. Published with the approval of the Director as Journal Series Paper Number 54.

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RESULTS

The following data show the development of internal cork in Porto Rico sweetpotatoes from plants which were exposed to aphid transmission and those protected from aphids.

Amount of Internal Cork	Aphid Transmission	Check
None	36	52
Light	6	0
Medium	7	0
Severe	4	1
Very Severe	0	0

The one case of internal cork in the checks may have been caused by some of the leaves of the plants in the cage of cork-free material touching the screen and a loose infective vector may have fed on the leaves through the screen.

On the basis of the above data, it is indicated that Myzus persicae is a vector of the internal cork virus.

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OBSERVATIONS ON LETTUCE MOSAIC IN CALIFORNIA

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Summary

Studies with the lettuce mosaic virus showed that its host range includes a number of previously unreported weed hosts, including dead nettle, chickweed, sowbane, lambsquarters, oxtongue, shepherds-purse, and others, many of which are prevalent in the lettuce-growing areas of the Salinas Valley, California. The susceptibility of these weeds was established in recovery tests from infected field plants or by inoculation of healthy seedlings in the greenhouse.

Lettuce mosaic-infected weeds were found in the vicinity of young lettuce fields and evidence indicates that these infected plants may serve as an additional source of virus for lettuce plants growing in areas adjacent to the weeds.

INTRODUCTION

Common mosaic is considered one of the major virus diseases of the lettuce plant (Lactuca sativa L.) in California (3). In the United States, as in Europe, diseased lettuce plants arising from mosaic-infected seed have been considered as the major source from which spread of the virus to healthy plants takes place (2, 3, 7, 8).

In attempts to determine the possible relationships of weed hosts to virus diseases of sugar beet, the occurrence of a rather high incidence of apparently virus-infected weed species in the areas of lettuce production in the Salinas Valley, an important lettuce growing area in California, was noted. Subsequent studies indicated that the lettuce mosaic virus was commonly found infecting these species. The host range of the lettuce mosaic virus, as reported in the literature, is rather limited and consists of only a few weed species and some cultivated plants. These facts led the writers to reinvestigate the host range of the lettuce mosaic virus with particular emphasis on the weed species that are prevalent in this area.

MATERIALS AND METHODS

Except in the case of virus recovery from field plants, the inoculations were performed mostly with lettuce mosaic virus obtained from lettuce seedlings grown from infected seed. Two lettuce varieties were used as test plants. Bibb, a variety that gives a typical mosaic reaction, and Eiffel Tower Cos, one that gives a necrotic response. In addition to lettuce seedlings, plants of Chenopodium amaranticolor Coste & Reyn. and sowbane (C. murale L.) were used as indicator hosts for the lettuce mosaic virus.

Both mechanical and insect inoculations were used in the experiments. Insect inoculations were carried out in most tests with the green peach aphid (Myzus persicae (Sulz.)) reared on radish (Raphanus sativus L.).

Diseased lettuce plants showing mosaic symptoms were used for feeding the non-viruliferous aphids for inoculations. In a few instances, infected groundsel (Senecio vulgaris L.) was also used as a virus source for the insects. The aphids were usually fed for a few hours on the virus source and then transferred onto the test plants and permitted to feed overnight.

Mechanical inoculations with the lettuce mosaic virus were carried out by routine methods using preparations obtained from infected leaves ground in 5 parts of 0.02M phosphate buffer at pH 7 in which sodium sulfite had been added to reach a 0.02M concentration. An abrasive was sprinkled on the leaves before inoculation. Other specific details are described with the experiments concerned.

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STUDIES ON HOST RANGE

Observations on host range were carried out: 1) by attempting to recover the lettuce mosaic virus from plant species growing in old lettuce fields where mosaic incidence had been high; 2) by inoculating a minimum of five potted seedlings of different species in the greenhouse, either mechanically or by means of the vector. Virus sources were considered infected by lettuce mosaic when mechanical or insect inoculations on plants of the Bibb and Eiffel Tower Cos varieties caused the mosaic and necrotic reactions, respectively, and when mechanical inoculation on Chenopodium amaranticolor caused local and systemic symptoms similar to those obtained with inoculum from seed-transmitted lettuce mosaic.

A list of the species that supported increase of the lettuce mosaic virus is given in Table 1. All represent hosts that appear not to have been reported previously as susceptible to this virus; the majority of the susceptible species, such as dead nettle, chickweed, sowbane, lambsquarters, oxtongue, shepherds-purse, and others, are common weeds in the Salinas Valley. Many of these weeds were found naturally infected with the lettuce mosaic virus.

Table 1. List of species from which the lettuce mosaic virus was recovered.

Anagallis arvensis L. (scarlet pimpernel) -- FR, GR[a]
Capsella bursa-pastoris (L.) Medic. (shephereds purse) -- FR
Chenopodium album L. (lambsquarters) -- FR, GR, S
C. amaranticolor Coste & Reyn. -- GR, S
C. ambrosioides L. -- GR, S
C. capitatum (L.) Asch. -- GR, S
C. murale L. (sowbane) -- FR, GR, S
Erodium cicutarium (L.) L'Her. (filaree) -- FR, GR, S
Lamium amplexicaule L. (dead nettle) -- FR, GR
Malva parviflora L. (cheeseweed) -- FR, GR, S
Nicotiana clevelandii Gray -- GR, S
Picris echioides L. (oxtongue) -- FR, GR, S
Silybum marianum (L.) Gaertn. (milk thistle) -- GR
Spinacia oleracea L. (spinach) -- GR, S
Stellaria media (L.) Cyr. (chickweed) -- FR, GR
Tetragonia expansa Murr. (New Zealand spinach) -- GR, S

[a] FR - Virus recovered from field plants;
GR - Virus recovered from greenhouse infected plants;
S - Symptoms shown by infected plants.

A number of other species were tested for susceptibility to lettuce mosaic virus in greenhouse experiments, but gave negative results. These included Nicotiana tabacum L. (tobacco), N. glutinosa L., Phaseolus vulgaris L. (bean), Datura stramonium L., Lycopersicon esculentum Mill. (tomato), Petunia hybrida Vilm (petunia), Beta vulgaris L. (sugar beet), Cynara scolymus L. (artichoke), and Sonchus oleraceus L.

SYMPTOMS CAUSED BY THE LETTUCE MOSAIC VIRUS ON SOME HOST PLANTS

Chenopodium amaranticolor: Leaves of this plant inoculated with the lettuce mosaic virus developed local lesions in about 4 to 5 days. The lesions were small chlorotic areas 1 to 3 mm in diameter and in most cases had a central necrotic spot. In plants growing rapidly, the systemic symptoms appeared 1 or 2 days later in the young leaves as circular chlorotic spots, sometimes elongated when occurring on a vein. A certain degree of crinkling was seen in the young diseased leaves. After the initial shock effect the systemic symptoms became less severe and there was a tendency for the lesions to become less numerous. Old local or systemic lesions consisted of reddish spots or lines, surrounded by chlorotic areas. Although many viruses cause local lesions on this host plant (5), most of them remain localized. The fact that the lettuce mosaic virus becomes systemic in C. amaranticolor provides a good distinction between this and many other viruses.

Chenopodium album L. (lambsquarters): The lettuce mosaic virus caused local and systemic symptoms on this species. They resembled those described for C. amaranticolor, but the reddish color was more noticeable in the old lesions.

Chenopodium murale L. (sowbane): Greenhouse seedlings of this species showed local and systemic chlorotic spots when infected with the lettuce mosaic virus. Late symptoms consisted of mottling and crinkling of the leaves. Infected plants in the field showed more severe symptoms. Mottling and crinkling were more distinct and leaf size was reduced. Infected field plants appeared bushier than normal owing to a shortening of internodes.

Sowbane is a common weed in the Salinas Valley and seedlings of this species infected by lettuce mosaic were found in great numbers in old lettuce fields in this area. Also, infected sowbane plants were encountered growing on roadsides near young lettuce fields. Prevalence of infected plants suggests that this weed may serve as a lettuce mosaic virus reservoir in this part of California.

Attempts were made to determine whether or not lettuce mosaic virus is transmitted through sowbane seeds. Over 500 seedlings of this species were grown in the greenhouse from seed harvested from plants infected by the lettuce mosaic virus. No seed transmission was detected as based on symptom observation and on recovery tests, but the results are not considered conclusive.

Malva parviflora L. (cheeseweed): Cheeseweed plants were infected with the lettuce mosaic virus by mechanical inoculation and by means of the vector. Infected plants showed a faint vein clearing and mild symptoms of mosaic. Since plants of this species are very common weeds on roadways near the lettuce fields in the Salinas Valley, it was thought that they might be important as virus reservoirs of lettuce mosaic. However, most of the attempts made to recover the lettuce mosaic virus from cheeseweed plants growing near young lettuce fields were negative. These results indicate that cheeseweed plants are either relatively resistant to lettuce mosaic under field conditions or that the virus disappears from the infected plants after the initial infection takes place. Evidence in respect of the latter hypothesis was obtained in greenhouse tests. It was found that the lettuce mosaic virus could be recovered from infected M. parviflora plants 15 days after inoculation, but recovery tests from the same plants were negative 30 days later.

Nicotiana clevelandii: Infected plants showed chlorotic spots or rings in the inoculated leaves. Systemic symptoms first appeared as a slight vein clearing accompanied by rugosity in the young leaves. Late symptoms consisted of yellowing of older leaves.

Spinacia oleracea L. (spinach): Spinach plants were easily infected with the lettuce mosaic virus by mechanical inoculation in the greenhouse. Early symptoms were not very distinct and consisted of a faint mottle. Late symptoms appeared as yellowing of the foliage, accompanied by some withering and death of leaves. The lettuce mosaic virus was readily recovered from infected spinach plants in the greenhouse as long as 3 months after inoculation.

Several attempts were made to recover the lettuce mosaic virus from field spinach plants, but they all failed. It was rather surprising that the lettuce mosaic virus could not be recovered from field-grown spinach plants in the Salinas Valley, since the chances of infection seem great owing to the great abundance of aphid vectors and numerous virus reservoirs.

DISCUSSION

The host range of the lettuce mosaic virus has been shown to include a number of common weeds, many of which may be found at all times of the year in the Salinas Valley. There is a possibility that these weed hosts may serve as additional sources of the lettuce mosaic virus. Several observations in the literature might be interpreted as suggesting that weed hosts may play a role in the spread of lettuce mosaic. In England (2) and in the United States (4), it was noticed that lettuce fields usually showed a higher incidence of mosaic near the edges than in the interior portions of the same fields. These results were attributed to a greater concentration of vectors in the border areas. Since weeds are more likely to occur in roadways and near hedges, fences, and buildings, it is possible that the higher incidence of lettuce mosaic near these places may have resulted, in part, from the migration of aphids that picked up virus from infected weed hosts.

The number of susceptible weeds infected by mosaic was high in some old lettuce fields in the Salinas Valley. This was particularly true of sowbane, one of the common weeds in this area. In the Salinas Valley where weeds are present the year around, the possibility exists that infected weed hosts will harbor the virus between two consecutive crops and so serve as additional virus sources. Of course, even in this case, the importance of weeds as virus reservoirs would be greater near the field borders and probably less so in the middle of large fields. Weed hosts would be of lesser importance in lettuce-growing areas where a severe winter will kill them and so break the cycle of the disease in the field.

The importance of weed hosts in relation to lettuce mosaic spread should also be considered from other viewpoints. It is possible that the lettuce mosaic virus is carried in the seeds of other weed species, such as groundsel (1), and so is perpetuated between growing seasons. Another point to be considered is that many weeds growing near the lettuce rows might become infected early in the season with virus from seed-infected lettuce plants and then supply virus back to the lettuce plants when weeding and thinning operations are carried out.

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EFFECT OF ROOT-KNOT NEMATODES ON FUSARIUM WILT OF MIMOSA[1]

D. L. Gill[2]

Abstract

Mimosa (Albizzia julibrissin) seedlings were grown in (a) uninfested soil, and in soil infested with (b) Fusarium oxysporum f. perniciosum, (c) Meloidogyne incognita or M. javanica, or (d) Fusarium together with either M. incognita or M. javanica. More wilting occurred in soil infested with Fusarium and either of the nematodes in combination than in soil infested with Fusarium alone. The nematodes alone reduced seedling size. M. javanica produced an abundance of root galls, while M. incognita produced a small number of root galls.

Nematodes have been found associated frequently with increased severity of plant diseases attributed to other causes (1). Root-knot nematodes (Meloidogyne spp.), as well as other nematodes in association with pathogenic species of Fusarium, have increased wilting. Martin, Newsom, and Jones (3) reviewed the previous work on the nematode-wilt complex of cotton and presented additional data. Jenkins and Coursen (2) reviewed briefly the work on association of nematodes with disease-producing organisms and presented further evidence that root-knot nematodes increased the susceptibility of tomatoes to Fusarium wilt. Morgan (4) reported control of both Fusarium wilt of tobacco and root-knot nematodes by fumigation for nematode control and reviewed the nematode-disease complex. Rankin (5) found that Fusarium wilt of okra was increased by root-knot and root-lesion nematodes.

The author knows of no report showing the effects of nematodes upon Fusarium wilt of a woody plant, but root-knot nematodes have been observed frequently on apparently healthy mimosa trees (Albizzia julibrissin Durazz.) and on trees wilted with Fusarium oxysporum f. perniciosum (Hepting) Toole. The occurrence of root-knot nematodes on mimosa trees dying from wilt suggests the possibility of a nematode-wilt complex. The three tests reported here were designed to determine whether root-knot nematodes affect Fusarium wilt of mimosa seedlings.

MATERIALS AND METHODS

The three tests were essentially the same and consisted of growing mimosa seedlings in soil given four different treatments. The soils were (a) uninfested, (b) infested with F. oxysporum f. perniciosum, (c) infested with root-knot nematode, and (d) infested with F. oxysporum f. perniciosum plus root-knot nematode. Three flats of soil 6 inches deep were used for each treatment, making 12 flats for each test. Except in Test 3, 100 seeds from a tree considered wilt-susceptible were planted in one half of each flat and 100 seeds of the wilt-resistant variety Tryon in the other half of each flat. In Test 3, 50 seeds from a wilt-resistant tree designated 27-65 BS(24) replaced 50 of the Tryon seeds. The number of susceptible seeds was the same as in the other tests.

On November 19, 1956, the different inoculums used in Test 1 were thoroughly mixed with the soils previously steam-sterilized in flats. Inoculum of Meloidogyne incognita (Kofoid & White) Chitwood[3] was obtained from galled tomato roots, thoroughly washed, and ground in a food chopper to facilitate mixing with the soil. The fungus inoculum consisted of four isolates of F. oxysporum f. perniciosum grown on potato-dextrose agar for 10 days and blended in a Waring Blendor.

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[1]Cooperative investigations at Tifton, Georgia, of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the University of Georgia College of Agriculture Agricultural Experiment Stations. Published with the approval of the Director as Journal Series Paper Number 53.

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[3]Nematode identification by J. M. Good, Jr., Nematologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

The flats of soil in Tests 2 and 3 were fumigated with methyl bromide. In Test 2 the seeds were planted January 23 and in Test 3 February 18, 1957. The susceptible seeds used in Test 3 were from a different source from those used in Test 2 because the latter germinated poorly.

Soil used in Tests 2 and 3 was infested May 1, 1957. The inoculum was placed on the surface of the soil to avoid root injury. A light covering of peat moss kept the inoculum moist. Fungus inoculum consisted of a mixture of eight isolates of the mimosa wilt fungus cultivated and handled as in Test 1. Inoculum of Meloidogyne javanica (Treub) Chitwood[3] was originally obtained from mimosa roots with no evidence of wilt symptoms, transferred to fumigated soil, and cultured on tomato roots. After small root-knot galls formed, the roots were washed and used to infest a second lot of fumigated soil. Tomato roots with small galls from this second lot of soil served as the inoculum in Tests 2 and 3. This procedure reduced the possibility of transmitting F. oxysporum f. perniciosum with the nematode inoculum.

In all three tests the flats were kept in a greenhouse until mid-April, when they were moved under pine-tree shade and supported off the ground. Flats with different treatments were spaced several feet apart.

RESULTS AND DISCUSSION

The effects of the soil infestations upon survival of mimosa seedlings from wilt-susceptible and wilt-resistant seeds are shown in Table 1 and Figure 1. The percentage of plants surviving in the uninfested soil was less than the percentage surviving in the soil infested with nematodes alone (Table 1). Plants dying in the uninfested soil were small and did not exhibit wilt symptoms.

FIGURE 1. Effect of Fusarium oxysporum f. perniciosum and Meloidogyne javanica upon mimosa seedlings: Seedlings growing in soils (a) uninfested, (b) infested with Fusarium, (c) infested with Fusarium plus nematodes, and (d) infested with nematodes, from upper left to lower right, respectively.

Table 1. Effects of Fusarium oxysporum f. perniciosum and/or Meloidogyne spp. upon survival of wilt-susceptible and wilt-resistant mimosa seedlings[a].

Test and soil inoculum	Number of plants of indicated reaction to wilt living on date shown						Percent of plants living on December 18, 1956 still alive on November 1, 1957		Percent inoculated plants still living on November 1, 1957	
	December 18, 1956		April 29, 1957		November 1, 1957					
	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Test 1--Soil infested Nov. 19, seeds planted Nov. 20, 1956:										
No inoculum	253	256	242	250	134	150	53	59		
M. incognita alone	160	184	158	180	136	125	85	68		
Fusarium alone	25[b]	86[b]	23[b]	80[b]	5[b]	26[b]	20	30		
M. incognita plus Fusarium	65	189	33	104	2	3	3	2		
Test 2--Seeds planted Jan. 23, soil infested May 1, 1957:										
No inoculum			7	168	7	97			100	58
M. javanica alone			35	166	17	122			49	73
Fusarium alone			15	169	10	66			67	39
M. javanica plus Fusarium			8	136	1	6			13	4
Test 3--Seeds planted Feb. 18, soil infested May 1, 1957:										
No inoculum			136	89[c]	102	67[c]			75	75
M. javanica alone			194	138[c]	175	121[c]			90	88
Fusarium alone			168	129[c]	51	67[c]			30	52
M. javanica plus Fusarium			163	137[c]	3	10[c]			2	7

[a]300 seeds from wilt-susceptible tree and 300 seeds from a wilt-resistant tree planted in each treatment and test.
[b]Plants from 200 seeds.
[c]Plants from 150 seeds.

The reduction in percentage of plants surviving in the uninfested soil is thought to be caused by the more vigorous growth of some seedlings crowding and killing the less vigorous ones. The mean heights of the Tryon seedlings growing in Test 3 on November 1, 1957 is representative. These heights were (a) soil uninfested 25.0 cm, (b) soil infested with M. javanica 14.5 cm, (c) soil infested with F. oxysporum f. perniciosum 16.3 cm, and (d) soil infested with Fusarium plus M. javanica 8.3 cm. Leaves of plants in the nematode-infested soil were lighter green and smaller than the leaves of control plants. Roots in the nematode-infested soils were heavily galled by M. javanica but only slightly galled by M. incognita.

A greater reduction in the percentage of seedlings surviving occurred in the soil infested with the combination of Fusarium and either M. incognita or M. javanica than in the soil infested with Fusarium alone. Plants in root-knot-nematode-infested soil did not exhibit wilt symptoms, while plants wilting in soil infested either with F. oxysporum f. perniciosum alone or with Fusarium plus root-knot nematodes exhibited typical wilt symptoms including vascular discoloration. Thus the presence of the root-knot nematodes, M. incognita or M. javanica, increased the incidence of Fusarium wilt of mimosa seedlings. However, the nematodes were not necessary for wilt production. This is in agreement with the results of other workers on root-knot-nematode-Fusarium-wilt complexes (1, 2, 3, 4, 5). With a woody plant such as mimosa it is probable that after several years the number of plants wilting in soil infested with Fusarium alone would not differ from the number wilting in soil infested with root-knot nematodes plus Fusarium, but the root-knot-infested plants would succumb more quickly.

The data do not permit a conclusion as to a difference in the behavior of the seedlings from the susceptible trees and those from the two resistant trees. This is not unexpected since resistant trees are not homozygous for resistance.

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UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, AND UNIVERSITY OF GEORGIA COLLEGE OF AGRICULTURE EXPERIMENT STATIONS

EXTENT OF SUSCEPTIBILITY WITHIN KENAF, HIBISCUS CANNABINUS L., TO ROOT-KNOT NEMATODES[1]

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Abstract

All kenaf varieties, introductions, and selections tested were susceptible to Meloidogyne incognita and M. incognita acrita, but some exhibited variation particularly between plants within lines. Individual selections tested exhibited more apparent tolerance than the parent lines from which the selections were made.

The heavy loss in yield of fiber when kenaf, Hibiscus cannabinus L., was grown on soil infested by the root-knot producing nematodes, Meloidogyne incognita and M. incognita acrita, suggested a study to determine the degree to which the available kenaf introductions and selections were resistant, tolerant, or susceptible to those nematodes[3]. That portion of the approach to the problem involving interspecific crosses between kenaf and resistant non-fiber type related species is being reported elsewhere.

A program of testing varieties, lines, and selections which showed agronomic promise was started in 1953 and continued through 1956. A total of 89 selections, introductions, and varieties was tested. The variation among lines and selections of kenaf, as measured in average tolerance or resistance, was not great. However, the variability of plants within lines and varieties was sufficient to warrant the initiation of a selection and breeding program designed to improve the degree of tolerance to M. incognita and M. incognita acrita, the most prevalent of the destructive root-knot nematodes attacking kenaf in Florida[4].

This report indicates the extent and degree of susceptibility existing within the presently available introductions and selections of Hibiscus cannabinus, which either appear to be adapted agronomically for fiber production in Florida, and/or possess some value as breeding material to be used in developing such adapted varieties.

A scoring scheme was utilized, with ratings from "1.00", which indicated no visible gallation, to "5.00", which indicated complete transformation of the root system into a mass of galls where the elongated root structure no longer existed. The five root systems shown in Figure 1 give an approximation of the rating classes.

Individual selections were made in 1956 from those lines and varieties which in previous years had given an indication of possessing some apparent tolerance or a wide range of variation in individual plant reaction to nematode attack. Figure 2 shows a portion of the nematode test area in 1956 with a few scattered living plants among many dead plants. Figure 3 illustrates the variation in plants and root systems that occurred within certain lines and varieties. This demonstrates the type material from which the 1956 selections were made. Later in the season, the test area was planted to the varieties Salvadorian and Cuba 108. The seed from the plants that produced seed were harvested, bulked, and planted as composites in 1957, along with seed from the parent lines and individual selections made in 1956.

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[1] Florida Agricultural Experiment Station Journal Series, No. 740. The research work on which this report is based was conducted cooperatively by the Crops Research Division and Agricultural Engineering Division, Agricultural Research Service, United States Department of Agriculture, and the Everglades Experiment Station of the University of Florida Agricultural Experiment Stations.

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[3] Summers, T. E., 1954. Fiber Crops Investigation. Annual Report, University of Florida Agricultural Experiment Stations. p. 219.

[4] Appreciation is expressed to Dr. B. G. Chitwood, State Plant Board, Gainesville, Florida for making identifications through morphological characters substantiating determinations of prevalence made through the use of differential plants.

FIGURE 1. Roots from left to right illustrate nematode ratings of "1.00" to "5.00" respectively.

FIGURE 2. General view of nematode test area. Note variation in plants ranging from living, apparently healthy plants to completely dead plants

FIGURE 3. Variation in plants within an individual row of Salvadorian kenaf. Selections from such rows were made in 1956 and first tested in 1957.

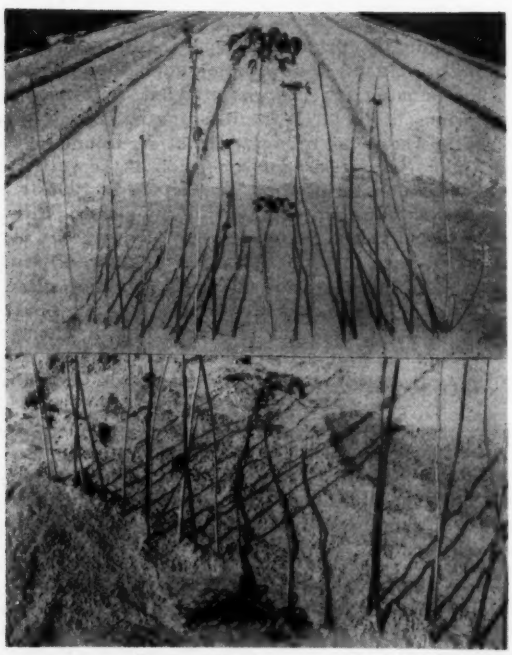

Table 1. Means and ranges of susceptibility to root-knot nematodes of certain kenaf selections and composites compared with parent varieties and lines from which the selections and composites were derived.

Parent variety or line	Selections or composites from parents	Root-knot nematode susceptibility ratings* 1957 Mean	1957 Range	1956 Mean	1956 Range	1953-54 Mean	1953-54 Range
Salvadorian		3.6	3-4	4.1	3-5	4.0	2-5
	BG-56-2	2.8	2-3				
	BG-56-3	2.6	2-3				
	BG-56-4	2.7	2-3				
	BG-56-5	2.3	2-3				
	BG-56-8	2.2	2-3				
	BG-56-12	3.3	3-4				
	BG-56-15	3.0	3-4				
	Sal. Composite	2.7	2-4				
Cuba		3.5	3-4	3.5	3-5		
	Cuba 108 Composite	2.5	2-4				
Cubano		3.0	2-4	3.9	2-5		
BG-52-65		3.8	3-5	3.6	2-5	4.0	2-5
	BG-56-1	2.0	2-2				
	BG-56-7	2.0	2-2				
	BG-56-9	2.0	2-2				
BG-52-71				4.4	3-5	5.0	5-5
	BG-56-6	2.5	2-3				
BG-52-75		3.6	3-4	3.9	2-5	4.0	2-5
	BG-56-10	3.1	3-4				
	BG-56-14	4.0	4-4				

*Means and ranges based on 1.0 to 5.0 susceptibility ratings of 10 plants, 1.0 being resistant and 5.0 highly susceptible.

Table 1 presents the over-all 1957 nematode rating, as well as the range of variability in parent lines, individual selections made in 1956, and plants from the bulk seed from the 1956 test area. Also indicated are the 1956 and 1953-54 rating and the range of variation for some of the lines tested. As seen in columns 3 and 4 of Table 1, the selections made in 1956, with one exception, have a better rating than the parent lines in 1957. Additional work with these selections may produce a line with less susceptibility to root-knot nematodes than previously available fiber types. Pure populations of the two species of nematodes involved have been established with the hope that resistance to the individual species may be found and subsequently combined into one variety.

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VIABILITY OF AIR-DRIED HETERODERA GLYCINES CYSTS[1]

James M. Epps[2]

Abstract

In two experiments, no viable larvae of the soybean cyst nematode, Heterodera glycines Ichinohe, were found in cysts after a 1-month storage period in seed bags. In one experiment live larvae were found in cysts stored with seed for only 1 month, and these produced white females on the roots of potted soybean plants. There were no viable larvae found in this experiment after storage for 2 and 3 months.

INTRODUCTION

The soybean cyst nematode, Heterodera glycines Ichinohe, 1952, was reported in North Carolina in 1955 (1). Since that time it has been found in Missouri (3), Arkansas, Kentucky, and Mississippi. Its presence in these States has created problems not encountered previously, since some growers in this region produce certified seed for sale in other areas, and there is a possibility of cysts becoming contaminants of the seed. In experiments in North Carolina, only a fraction of 1 percent of larvae in cysts survived air drying in open dishes for 6 weeks (4). Results of experiments on survival of larvae in cysts stored with seed are presented.

MATERIALS AND METHODS

Experiment No. 1: On August 19, 1957, three bags of soybean seed were stored, each under a different storage condition. One bag was stored in the greenhouse where there was a wide range of temperature variations, ranging from approximately 70° to 95° F. One bag was stored in a garage where the temperature did not vary as much and rarely reached 85° on the hottest days. A third bag was stored in an air conditioned building where the temperature ranged from 70° to 80°. To each of these bags of seed two kinds of cyst material were added. One kind consisted of 24 small muslin bags of cysts, each bag containing the cysts extracted from a 2-pound soil sample by sieving, the cysts being retained on a 60-mesh sieve. These were free of soil, but were mixed with debris which was also retained by the sieve. Another lot of 24 bags of cysts from 2-pound soil samples was added, and to each of these samples some soil was added. Thus each large bag of seed contained 24 small muslin bags of cyst material without soil, and 24 small bags of cyst material plus some soil. Each of the bags contained several hundred cysts with eggs and viable larvae.

After 1 month of storage, eight small bags of each kind of cyst material were removed from each bag of seed. A total of 150 cysts were separated, and the contents examined to determine if there were live larvae in the cysts. About half of the remaining cyst material was placed on Baerman funnels and allowed to remain for 10 days. The other half was placed in 6-inch clay pots and planted to Ogden soybeans. Approximately 30 days after the soybeans were planted, the plants were removed from the pots and the roots were washed and examined for the presence of white females. After examination, the roots were stained in Acid Fuchsin and examined to determine if larvae had entered the roots. Eight more bags of each kind of cyst material were removed after 2 months' storage and the remaining eight bags after 3 months' storage, the above examination procedure being repeated each time.

On examination at the end of 1, 2, and 3 months, no viable larvae were found in the cysts nor in the Baerman funnels. No white females were found on the roots, nor were any larvae found in the stained roots. Under the conditions of this test, the contents of the cysts were not viable after one month of storage.

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[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Tennessee Agricultural Experiment Station.
[2]Nematologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Jackson, Tennessee.

Experiment No. 2: This experiment differed from experiment No. 1, in that the cysts were mixed with the seed. Only one bag of seed was used and storage was in a garage where the temperature was rarely over 85° F. Cyst material was obtained by the flotation method and collected on a 60-mesh seive. These cysts were mixed with damp soil. The soil and cyst material were placed in a small drum with the seed and rotated until the seed were well covered with the damp soil containing the cysts. The bag of seed was placed in storage on August 19, 1957. At monthly intervals for 3 consecutive months, a volume of seed was removed and the cysts separated. The cysts were examined for live larvae. Some of the material was then put in 6-inch clay pots and planted to soybeans. One month after planting, the plant roots were washed and examined for white females, and then stained as in experiment No. 1.

The results of this test were the same as in experiment No. 1. None of the cysts were found to contain viable larvae after 1 month in storage, nor were white females and larvae found on or in the roots.

Experiment No. 3: In experiment No. 3 large numbers of cysts washed from soil were mixed with soil and debris collected from three large combines that were being used to combine soybeans in infested fields. This material was placed in nine large clay saucers and stored in three separate buildings similar to average buildings in which seed might be stored on a farm. Three saucers were stored in each of the buildings.

At monthly intervals for 3 consecutive months, one saucer from each of the buildings was brought to the laboratory. A large number of cysts was separated and the cyst contents examined for the presence of viable larvae. The remainder of the cyst material was put in 4-inch clay pots and planted to soybeans. The roots of the plants were washed and examined for white females 30 days after planting. The roots were stained and examined to determine if any larvae had entered the roots.

Live larvae were found in one of the storage buildings 1 month after storage, and when the material was placed in pots and planted to soybeans, white females developed on the roots. In the other two storage buildings, no live larvae were found at the end of 1 month. There were no live larvae observed in any of the saucers at the end of 2 months, nor did white females develop on soybean roots in pots to which the cyst materials had been added. Thus under two of the three storage conditions, no live larvae were found at the end of 1 month of storage while under the third storage condition, live larvae were found at the end of 1 month of storage, but not after 2 months.

DISCUSSION

Under the conditions of one of these tests, the cysts contained viable larvae after storage for 1 month. Under the other conditions, no viable larvae were found at 1, 2, and 3 months after storage. In no case did white females develop on the roots of soybean plants when grown in cyst material that had been stored for 2 months.

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REPRODUCTION OF MELOIDOGYNE SPP. IN FLUE-CURED TOBACCO LINES OF ROOT-KNOT RESISTANT PARENTAGE[1]

P. N. Drolsom and E. L. Moore

Summary

Root-knot resistant breeding lines of flue-cured tobacco (Nicotiana tabacum) were grown individually with two or more species of Meloidogyne. Observations on egg mass formation are presented. Data indicated that breeding lines were highly resistant in the presence of M. incognita and M. incognita acrita and relatively susceptible with M. javanica, M. arenaria, and M. hapla.

The root-knot disease of flue-cured tobacco (Nicotiana tabacum L.), caused by nematodes of the genus Meloidogyne Goeldi, has been recognized for a long time as economically important. In the United States control has been based upon rotation and soil fumigation. The use of resistant varieties as an additional control measure would be very desirable. Clayton and Foster (1) reported root-knot resistance in cultivated tobacco in 1940. Recently Clayton et al. (2) described the problems encountered in combining this resistance with flue-cured type. Their testing of breeding lines for root-knot resistance was done primarily with M. incognita (Kofoid & White) Chitwood and (or) M. incognita acrita Chitwood, the most prevalent forms in the flue-cured tobacco producing area.

The differential response of plants when attacked by individual species of Meloidogyne is well known (5, 6, 7, 8). Since the root-knot resistant breeding lines have great potential value in the flue-cured varietal improvement program it appeared desirable to have information about their reaction to different Meloidogyne species. A preliminary report (3) indicated that resistance in flue-cured tobacco was not equally effective against M. incognita, M. incognita acrita, M. javanica (Treub) Chitwood, M. arenaria (Neal) Chitwood, and M. hapla Chitwood. This investigation was continued, and the reaction of 13 lines to these root-knot nematode species is summarized herein.

MATERIALS AND METHODS

Plants of breeding lines with root-knot resistance as outlined by Clayton et al. (2) were grown in steam-sterilized soil to the four- or five-leaf stage. At that time the bulk of soil was washed from the roots before transplanting to root-knot infested soil in 4-inch clay pots. Inoculum for each plant consisted of 4 to 5 grams of chopped tomato or tobacco roots bearing egg masses. A minimum of four plants of a breeding line was grown with each nematode species. Sixty to 70 days after inoculation the roots were washed and rated for gall development, root decay, and egg mass production. Representative data are presented for the latter, because egg formation denotes reproduction in the host whereas gall development does not mean necessarily completion of the life cycle. However, egg development always followed gall formation in the breeding lines studied.

Based upon the relative number of egg masses present, plants were placed into one of five classes: 0 -- no eggs visible, 1 -- trace, 2 -- moderate, 3 -- intermediate, and 4 -- numerous. Class values of 0, 10, 50, 80, and 100, respectively, were assigned each class. Egg mass indices were calculated by summation of class values x number of plants in each class, divided by total number of plants.

RESULTS

The breeding lines differed strikingly in the amount of egg production with various root-knot species (Table 1). There was little or no egg production by M. incognita and M. incognita acrita, whereas with M. javanica, M. arenaria, and M. hapla, eggs were abundant. Eggs

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[1] Appreciation is expressed to Dr. J. N. Sasser, North Carolina State College, for providing the original cultures of root-knot species, and for verification of species identifications during the investigation.

Table 1. Egg mass indices for 13 F5 and F6 breeding lines of tobacco grown with root-knot nematode species.

Breeding lines	Index with Meloidogyne spp.[a]				
	incognita acrita	incognita	javanica	arenaria	hapla
Bel 5-36-7[b]	26	—	72	62	—
" 5-36-17[c]	0	2	19	31	56
" 5-36-25	2	—	56	50	—
" 5-46-4	0	16	77	85	—
" 5-47-12	0	0	77	90	—
" 5-49-18	—	0	84	—	—
" 5-49-21	0	0	—	74	—
" 5-51-17[d]	0	—	54	84	32
" 5-53-2[d]	0	—	94	92	48
" 5-53-9	16	0	60	78	—
" 5-53-21	0	0	74	90	—
" 6-28	--	0	92	96	62
" 6-31	--	0	96	88	82
Susceptible varieties[e]	92	74	92	95	86

[a] Index computed from 4 or 5 plants, except as noted: [c] = 5 to 14 plants, [d] = 5 to 10 plants, and [e] = 10 to 20 plants.

[b] Bel 5 and Bel 6 lines received in 1955 and 1956, respectively, from the Tobacco Section, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

production generally was greater and decay was more evident in roots of susceptible check plants, indicating some resistance in the breeding material to all root-knot species.

Some lines, particularly in the 5-36 group, showed more resistance to M. javanica and M. arenaria than others. On the other hand, 5-36 lines often showed limited egg formation with M. incognita and M. incognita acrita, that is, one or two plants in a set of five would contain some eggs. Since plants of 5-36 were in the fifth generation and resistance to this population of M. incognita acrita is simply inherited (4), the continued occurrence of susceptible plants was not expected.

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A SURVEY OF PLANT PARASITIC NEMATODES IN PERU

L. R. Krusberg and Hedwig Hirschmann[1]

Summary

A survey of plant parasitic nematodes in several agricultural areas of the coast, mountains, and selva of Peru was made during July and August of 1957. A total of 208 collections contained 33 plant parasitic nematode species representing 14 genera. Meloidogyne spp. were most important on the coast and in the selva, and Heterodera rostochiensis in the mountains. Other genera found in the three geographic areas, often in large populations, were Tylenchorhynchus, Pratylenchus, Trichodorus, Helicotylenchus, and Rotylenchus. Genera occurring less frequently and in smaller populations were Xiphinema, Criconemoides, Paratylenchus, Tylenchulus, Hemicycliophora, Hemicriconemoides and Hoplolaimus.

Plant parasitic nematodes have in recent years been acknowledged throughout the world as important factors in crop production. Nematodes were previously recognized as economically important in Peru. In 1952 the golden nematode of potato, Heterodera rostochiensis, was found to be widespread in the mountains of Peru (25). Root-knot was observed on coffee in the selva (7) and a morphological study of Meloidogyne incognita acrita attacking tomato was made in Lima (13). The senior author spent June, July and August of 1957 in Peru as a phytonematologist in connection with the International Cooperation Administration. The purpose of the assignment was two-fold: first, to present a short course in plant parasitic nematodes for Peruvian agriculturists and, second, to make a nematode survey of several agricultural areas. The survey was conducted during July and August, which is the fallow season for the coast and mountain agricultural regions. The plant parasitic nematodes found during the survey are herein reported.

The survey included three geographical areas, the coastal irrigated valleys (altitude 0-700 feet), the mountains (altitude 8,000-14,000 feet) and the selva, or jungle, (altitude 1,800-4,800 feet). The specific areas sampled were around Lima and the Cañete Valley on the coast, Arequipa, Puno, Huancayo, Tarma, and Huaras in the mountains, and Tingo Maria in the selva (Figure 1). The economic importance of a particular crop determined the number of samples taken from that crop.

Soil samples of approximately 1 pint were collected, using a 1-inch diameter soil sampling tube. Each sample was a composite of scattered borings from a field. The soil was stored in quart polyethylene bags until processed. The method of Christie and Perry (4) was used to separate the nematodes from the soil. Cysts were caught on a 60-mesh screen and the eel-shaped nematodes on a 270-mesh screen. Species were determined from representative samples using specimens fixed in 1% formaldehyde.

RESULTS

The data are arranged according to geographic area and the relative economic importance of the nematode. A short description of the climate, soils and crops is included in the discussion of each area. Table 1 presents the comparative distribution and prevalence of plant parasitic nematode species found in the survey and the frequency and plants associated with the nematodes.

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FIGURE 1. Regional map of Peru.
A = elevation 0 - 656 ft., coastal area;
B = elevation 656 - 6562 ft.;
C = elevation 6562 - 16406 ft., mountain area;
D = elevation 656 - 6562 ft., selva;
E = elevation below 656 feet.
The number of samples (in parenthesis) and elevation are indicated for each sampling place. The 2 dotted ovals represent extensive sampling areas.

A. The Coast

The coastal regions of Peru have no rainfall. Due to the Humboldt Current a cloud bank envelops the coast from May until October resulting in high relative humidity. Most crop production is suspended during this period since the streams from coastal mountains do not supply sufficient irrigation water. Crops are only grown near rivers where limited irrigation water is available. The temperature during the year varies from 10 to 30° C with a mean around 19° C. The soil is generally a dark silt loam. The principal crops are sugar cane and cotton, with a considerable production of corn, banana, citrus and vegetables. The 20 samples collected on the coast were from the following crops: cotton-7; alfalfa-3; pine-3; corn-2; tomato-2; banana-1; wheat-1; and potato-1.

Meloidogyne spp. (21) -- M. incognita (Kofoid & White) Chitwood, 1949 and M. incognita acrita Chitwood, 1949 are prevalent, and often mixed, in the coastal irrigated valleys. Both are limiting in the production of cotton. These nematodes are also serious on tomato, potato and other vegetables. Larvae were found in several soil samples, but no galls were present on the roots of the associated plants.

Heterodera spp. (6, 9) -- One larva of a Heterodera sp. was isolated from a sample collected at the La Molina Agricultural Experiment Station near Lima. No cysts were observed. It is interesting to note that H. rostochiensis Wollenweber, 1923 was not found in the coastal valleys although it is prevalent in the mountains and has undoubtedly been introduced to the coast many times.

Pratylenchus spp. (17) -- A few specimens of P. thornei Sher & Allen, 1953 were associated with tomato at La Molina. Small numbers of P. coffeae (Zimmermann) Sher & Allen, 1953 were associated with cotton and large numbers of P. brachyurus (Godfrey) Filipjev & Stekhoven, 1941 with corn in the Cañete Valley.

Tylenchorhynchus spp. (1) -- Large numbers of T. capitatus Allen, 1955 were associated with tomato at La Molina.

Helicotylenchus spp. (10, 19) -- A few specimens of H. erythrinae (Zimmermann) Golden, 1956 were present in samples from cotton at La Molina and with pine at Huarmey.

Trichodorus spp. (2) -- About 100 specimens of T. christiei Allen, 1957 were found in a sample of soil from alfalfa at the Irrigacion de la Joya located 40 miles west of Arequipa. None were encountered nearer the coast.

Xiphinema spp. (24) -- Nematodes of this genus were frequently found in samples from the coast. X. americanum Cobb, 1913 was associated with tomato and cotton, and probably the same species was present in the other samples in small numbers. In soil samples containing pine roots from Huarmey, thousands of X. americanum were recovered and the pine roots were severely galled.

Criconemoides spp. (16, 20) -- A few specimens of C. mutabile Taylor, 1936 were associated with tomato and alfalfa and some unidentified species with corn and pine.

B. The Mountains

In the mountains variable amounts of rainfall occur from December to March and there is very little cloudy weather. The Arequipa Valley has little rainfall and is entirely irrigated, while the agriculture in Puno, Huancayo, and Huaras depends mostly on natural precipitation. Tarma, although similar to Huancayo, is extensively terraced and irrigated. In Arequipa the temperature varies from 0° to 30° C with a mean around 16° C. The soil temperature remains below 15° C. The temperature in the other mountain areas varies with the altitude, Puno being the coldest at 12,500 feet elevation. The soil in Arequipa is a dark silt loam similar to that at Lima, while in the other mountain areas it varies from a stony light-colored loam to stony red clay. The irrigated areas are used chiefly for the production of vegetables and alfalfa. while the non-irrigated areas are planted to potato, corn and wheat. The latter three are the principal crops. The 127 samples collected in the mountains were from the following crops: potato-43; wheat-14; garlic-12; onion-12; alfalfa-11; barley-9; corn-7; fruit trees-5; oats-3; eucalyptus-3; carrot-2; fallow-2; habas-2; pasture-1; and citrus-1. Although the time of sampling was in the middle of the dry season in the mountains and the soil was extremely hard and powdery dry, large populations of various plant parasitic nematodes were frequently encountered.

Meloidogyne spp. (21) -- In the Arequipa Valley Meloidogyne larvae were present in 26 of the 51 samples, varying in number from a few to about 200 per sample; however, galls were not found on the plants and the species could not be identified. No Meloidogyne larvae were found in the Huancayo or Tarma areas, but small numbers were associated with crops in the Huaras area.

Heterodera spp. (6, 9) -- H. rostochiensis is probably the most important plant parasitic nematode occurring in the mountains. Usually only a few cysts were found, but in some soil samples there were several hundred. Cysts of this species were recovered in 20 of the 51 samples from the Arequipa Valley, all 4 samples from Puno, 10 of 35 samples from Huancayo and Tarma and 15 of 37 samples from the Huaras area. Larvae were usually present only in samples from fields that had just produced potatoes, or were planted to potatoes the previous year. Potato is grown every 5 or 6 years in the rotations used in Arequipa. Cysts of H. rostochiensis were present in soil samples from the following crops in Arequipa: onion-8; garlic-6; potato-2; wheat-2; and alfalfa-1.

Puno had the most concentrated population of cysts of H. rostochiensis encountered in Peru. One field planted to potato 7 years previously, and since planted to continuous pasture plants contained many cysts, often with eggs. In the Huancayo, Tarma and Huaras areas crop rotations vary from consecutive potatoes to potato only once every 7 years with usually 2 years of barley or other small grain and 5 years fallow in between. Most rotations include potato every second to fourth year. Cysts of H. rostochiensis were present in soil samples from the following crops from the Puno, Huancayo, Tarma and Huaras areas: potato-12; barley-5; wheat-3; oats-2; alfalfa-1; corn-1; and fallow-1.

Cysts and larvae of H. schachtii were identified from samples collected in Arequipa. In 19 of the 50 samples collected from this area, lemon-shaped cysts occurred frequently in small numbers. In 9 samples, both these cysts and cysts of H. rostochiensis were present. Lemon-shaped cysts were also found around Tarma and Huaras, in 8 of 46 samples. Larvae in a sample from Huaras containing such cysts were identified as H. major (Schmidt) Franklin, 1940. Crops with which the cysts of the schachtii group were associated were: garlic-9; potato-7; onion-4; alfalfa-4; eucalyptus-1; carrot-1; wheat-1; bean-1; and citrus-1.

Pratylenchus spp. (17) -- Nematodes of this genus were present in 102 of 127 samples from the mountain region. The following species were identified from 26 of the samples: P. minyus Sher & Allen, 1953, P. thornei, P. penetrans (Cobb) Filipjev & Stekhoven, 1941, P. coffeae and P. pratensis (de Man) Filipjev, 1934. Two species were often present in the same sample. There were from a few to 400 nematodes per soil sample with the average around 100.

Tylenchorhynchus spp. (1) -- Tylenchorhynchus spp. were also found in 102 of 127 soil samples. Usually both Tylenchorhynchus spp. and Pratylenchus spp. were present in the same sample. The following Tylenchorhynchus species were identified from 24 samples: T. capitatus, T. brevidens Allen, 1955, and T. dubius (Bütschli) Filipjev, 1936. These were found associated with various plants, but the largest populations tended to be present where small grains were planted in 1957 or the previous year. From a few to 500 nematodes were present in a sample, the average population being 200.

Helicotylenchus and Rotylenchus spp. (10, 14, 18, 19) -- Only 6 of the 51 samples from the Arequipa Valley contained spiral nematodes. Rotylenchus boocki Lordello, 1957 was identified from five samples, three from fruit trees and one each from eucalyptus, barley and alfalfa. One sample from fruit trees also contained R. robustus (de Man) Filipjev, 1936. Spiral nematodes, generally in small populations, were present in 11 of 16 samples from near Tarma, and R. robustus and Helicotylenchus erythrinae were identified from 2. Spiral nematodes were most widely distributed in the Huaras area -- in 29 of 37 samples containing populations varying from a few to 300 nematodes per sample. R. robustus, R. boocki, and H. erythrinae were identified from 9 samples.

Trichodorus spp. (2) -- Nematodes of this genus were present in 33 of the 51 samples collected in the Arequipa Valley and in 6 of 37 samples from the Huaras area. Species were identified from 10 samples from Arequipa and 9 samples contained T. primitivus (de Man) Micoletzky, 1922 and 2, T. christiei; 2 samples from Huaras contained T. monohystera Allen, 1957. Populations varied from a few to 500 nematodes per sample.

Xiphinema spp. (24) -- Specimens of this genus were present in only four samples from the mountains. X. americanum was identified from two samples. Only a few Xiphinema nematodes were present in each sample.

Criconemoides spp. (16, 20) -- Small populations of these nematodes were present in 15 of

the 127 samples from the mountains. Specimens from four samples were identified as C. mutabile.

Paratylenchus spp. (11, 12, 15) -- A few specimens of P. macrophallus (de Man) Goodey, 1934 were found in two samples from fruit trees in Arequipa and some P. minutus Linford, Oliviera & Ishii, 1949 from a peach nursery in Huaras.

Hemicycliophora spp. (23) -- Small populations of H. typica de Man, 1921 were present in eight samples in Arequipa associated with various plants.

Hoplolaimus spp. (22) -- H. uniformis Thorne, 1949 was identified from one sample from fruit trees in Arequipa.

C. The Selva

The selva, around Tingo Maria, has about 140 inches annual precipitation and drainage is necessary for most crops. The growing season extends from May to October, the remainder of the year being too wet for crop production. The air is constantly humid. The temperature ranges from 15° to 35° C with the mean around 25° C. The area is mountainous and the soil a dark silt loam. The principal crops are banana, rubber, coffee and tea. The 61 samples from the selva were collected from the following plants: coffee-12; tea-12; citrus-11; pastures-7; cacao-5; rubber-4; banana-2; pimiento-2; pepper-1; tomato-1; corn-1; oil palm-1; barbasco-1 and pineapple-1.

Meloidogyne spp. (21) -- M. incognita acrita in tomato and pepper seedbeds and M. exigua Goeldi, 1887 on coffee and tea appeared to be the most serious nematode problems in the selva. Roots of plants in 6 of 12 coffee plantings examined were galled or Meloidogyne larvae were present. Among 11 samples from tea most contained larvae, but only a few galls and females were available for species identification. Only larvae were present in three samples from citrus, and in single samples from banana, cacao, rubber and pasture.

Heterodera spp. -- None observed.

Pratylenchus spp. (17) -- Nematodes of this genus were found in 7 of the 61 samples from the selva. P. brachyurus was identified from 4 samples and one also contained P. thornei. Populations were small.

Tylenchorhynchus spp. (1, 8) -- T. martini Fielding, 1956 was identified in 3 of 9 samples containing nematodes of this genus. Fairly large populations were associated with 5 samples from pastures and one from corn. A few nematodes of this genus were also present in single samples from rubber, coffee and citrus.

Helicotylenchus spp. (10) -- Helicotylenchus spp. were present in 54 of the 61 samples from the selva. Only H. erythrinae was identified from 17 samples. Populations were highly variable, from a few to 400 nematodes per sample. The average number of nematodes per sample was about 200. Perennial plants tended to be associated with larger populations.

Trichodorus spp. (2) -- Nematodes of this genus were found only in samples from perennial crops, and were present in 27 of the samples examined. T. monohystera and T. christiei were identified in 8 samples. Only small populations were found.

Xiphinema spp. (24) -- Xiphinema spp. occurred in 21 samples. X. americanum, X. radicicola Goodey, 1936 and X. chambersi Thorne, 1939, were identified from 7 samples. The nematodes were all associated with perennial crops and the largest numbers were found with coffee, citrus and tea.

Criconemoides (16, 20) and Hemicriconemoides spp. (3) -- These genera were present in 17 samples, always in small numbers. Identification to species has not been completed.

Paratylenchus sp. (11, 15) -- A few specimens of P. macrophallus were present in single samples from cacao, coffee, and barbasco.

Tylenchulus sp. (5) -- Females of T. semipenetrans Cobb, 1913 were abundant on roots of two citrus samples, one from Chanchamayo and one from Tingo Maria. In both cases large numbers of larvae and males were present in the surrounding soil.

Table 1. Distribution and relative prevalence of species of plant parasitic nematodes, and associated plants in Peru in 1957.

Number of samples examined	COAST 20		MOUNTAINS 127		SELVA 61		TOTAL 208
GENUS	Species	Crops and frequency[a]	Species	Crops and frequency[a]	Species	Crops and frequency[a]	
MELOIDOGYNE	incognita	Cotton-1 Tomato-2	(larvae only)	Onion-7 Alfalfa-7 Wheat-6 Potato-6 Garlic-4 Corn-2 Eucalyptus-1 Barley-1	incognita acrita	Tomato-1 Pepper-1	
	incognita acrita	Cotton-3 Tomato-2 Potato-1			exigua	Tea-3 Coffee-2	
	sp. undet.	Cotton-1 Corn-1 Wheat-1 Alfalfa-1			sp. undet.	Tea-5 Coffee-4 Pimiento-1 Banana-1 Cacao-1 Rubber-1 Pasture-1	
Totals	Total = 10		Total = 34		Total = 24		68
HETERODERA	(larva only)	Tomato-1	schachtii	Carrot-1 Garlic-1			
			major	Potato-1			
			rostochiensis	Potato-16 Onion-8 Garlic-6 Wheat-5 Barley-5 Oat-3 Alfalfa-2 Eucalyptus-1 Pasture-1 Fallow-1 Corn-1			
			schachtii type cysts	Garlic-8 Potato-6 Alfalfa-4 Onion-4 Eucalyptus-1 Wheat-1 Bean-1 Citrus-1			
Totals	Total = 1		Total = 69		Total = 0		70
PRATYLENCHUS	thornei	Tomato-1	thornei	Potato-4 Garlic-3 Barley-2 Onion-2 Pasture-1 Habas-1 Fallow-1	thornei	Pasture-1	
	coffeae	Cotton-1			brachyurus	Pasture-2 Coffee-1 Pineapple-1	
	brachyurus	Corn-1					
	sp. undet.	Tomato-1 Banana-1 Corn-1 Potato-1 Alfalfa-1 Cotton-1			sp. undet	Pasture-2 Rubber-1	
			coffeae	Potato-2			
			minyus	Potato-7 Garlic-3 Alfalfa-3 Barley-2 Habas-1 Fallow-1 Fruit trees-1 Wheat-1 Corn-1			
			penetrans	Potato-2 Fruit trees-1 Pasture-1			
			pratensis	Potato-1			
			sp. undet.	Potato-27 Wheat-12 Onion-9 Garlic-8 Alfalfa-7 Corn-5 Barley-4 Oat-2 Fruit trees-2			
Totals	Total = 9		Total = 102		Total = 7		118
TYLENCHORHYNCHUS	capitatus	Tomato-1	capitatus	Potato-7 Barley-2 Corn-2 Wheat-1 Alfalfa-1 Garlic-1 Fruit trees-1	martini sp. undet.	Pasture-2 Corn-1 Pasture-3 Rubber-1 Coffee-1 Citrus-1	
			brevidens	Barley-4 Potato-3 Onion-2			

Table 1. (Continued).

GENUS	COAST		MOUNTAIN		SELVA		TOTAL
	Species	Crops and frequency[a]	Species	Crops and frequency[a]	Species	Crops and frequency[a]	
			brevidens	Alfalfa-1 Garlic-1 Carrot-1 Fruit trees-1			
			dubius	Potato-1			
			sp. undet.	Potato-27 Wheat-11 Onion-11 Garlic-8 Alfalfa-7 Barley-3 Corn-3 Fruit trees-3 Oats-2 Pasture-1 Fallow-1 Eucalyptus-1			
Totals	Total = 1		Total = 102		Total = 9		112
HELICOTYLENCHUS and ROTYLENCHUS	H. erythrinae	Cotton-1 Pine-1	H. erythrinae	Potato-4 Corn-1 Fruit trees-1	H. erythrinae	Tea-4 Citrus-4 Coffee-3 Pasture-2	
			R. boocki	Fruit trees-3 Potato-1 Alfalfa-1 Eucalyptus-1		Cacao-1 Corn-1 Oil palm-1 Pineapple-1	
			R. robustus	Potato-5 Corn-2 Fruit trees-1	sp. undet.	Coffee-10 Tea-7 Citrus-6 Cacao-5	
			sp. undet.	Potato-9 Wheat-6 Barley-4 Corn-4 Alfalfa-2 Oat-2 Carrot-1 Fallow-1		Rubber-4 Banana-2 Pimiento-2 Tomato-1 Pasture-1 Barbasco-1	
Totals	Total = 2		Total = 46		Total = 54		102
TRICHODORUS	christiei	Alfalfa-1	christiei	Wheat-1 Alfalfa-1	christiei	Coffee-1 Citrus-1	
			primitivus	Garlic-3 Alfalfa-2 Fruit trees-2 Onion-1 Wheat-1	monohystera	Tea-3 Coffee-2 Citrus-1	
			monohystera	Potato-1 Corn-1	sp. undet.	Citrus-5 Tea-5 Coffee-3 Cacao-2 Pimiento-1	
			sp. undet.	Garlic-8 Potato-7 Onion-6 Wheat-3 Alfalfa-2 Carrot-1		Pasture-1 Barbasco-1	
Totals	Total = 1		Total = 39		Total = 27		67
XIPHINEMA	americanum	Pine-3 Tomato-1 Corn-1	americanum	Fruit trees-1 Potato-1	americanum	Citrus-3 Coffee-1	
	sp. undet.	Cotton-4 Tomato-1 Wheat-1 Alfalfa-1	sp. undet.	Fruit trees-1 Pasture-1	radicicola	Pimiento-1 Coffee-1	
					chambersi	Pineapple-1	
					sp. undet.	Coffee-5 Tea-3 Pasture-2 Cacao-1 Corn-1 Citrus-1 Rubber-1	
Totals	Total = 12		Total = 4		Total = 21		37

Table 1. (Concluded).

GENUS	COAST		MOUNTAINS		SELVA		TOTAL
	Species	Crops and frequency[a]	Species	Crops and frequency[a]	Species	Crops and frequency[a]	
CRICONEMOIDES and HEMICRICONEMOIDES	C. mutabile	Tomato-1 Alfalfa-1	C. mutabile	Fruit trees-1 Wheat-1 Alfalfa-1	Criconemoides sp.	Pasture-1 Tea-1 Coffee-1	
	sp. undet.	Corn-1 Tomato-1 Pine-3	sp. undet.	Potato-4 Fruit trees-2 Onions-2 Eucalyptus-1 Alfalfa-1 Garlic-1 Barley-1	Hemicriconemoides sp. sp. undet.	Cacao-1 Coffee-4 Citrus-2 Tea-2 Cacao-1 Pimiento-1 Pasture-1 Barbasco-1 Rubber-1	
Totals	Total = 7		Total = 15		Total = 17		39
PARATYLENCHUS			macrophallus	Fruit trees-1	macrophallus	Cacao-1 Coffee-1	
			minutus	Fruit trees-1			
			sp. undet.	Fruit trees-1	sp. undet.	Barbasco-1	
Totals	Total = 0		Total = 3		Total = 3		6
HEMICYCLIOPHORA			typica	Wheat-2 Fruit trees-2 Onion-1 Garlic-1 Eucalyptus-1 Alfalfa-1			
Totals	Total = 0		Total = 8		Total = 0		8
TYLENCHULUS					semipenetrans	Citrus-2	
Totals	Total = 0		Total = 0		Total = 2		2
HOPLOLAIMUS			uniformis	Fruit trees-1			
Totals	Total = 0		Total = 1		Total = 0		1

[a]The crops opposite species names indicate samples in which nematodes were identified to species. The number following the crop name is the number of times the nematode species was identified from samples of that crop. Samples in which only the genus was identified are shown by sp. undet. The totals are the sums of the samples in which nematodes of the genus were present.

DISCUSSION

Although this survey was much too restricted to draw any definite conclusions about ecological factors that influence the distribution of the different genera and species of plant parasitic nematodes occurring in Peru a few remarks on distribution trends of some species are of interest.

One Heterodera larva was present in a sample from the coastal area and this genus was totally absent in the selva, while in the mountains Heterodera spp. were among the most prevalent plant parasitic nematodes. The genus Meloidogyne on the other hand seems to play an important role in the coastal area and in the selva, whereas in the mountains the development of Meloidogyne spp. appears to be rather restricted. The alternating distribution pattern of these two genera may largely be attributed to the climatic differences of the three areas surveyed. Most of the members of the genus Heterodera, particularly H. rostochiensis which is of main importance in the mountain area, are known to be cold weather organisms, whereas Meloidogyne spp. thrive best under subtropical and tropical conditions as they exist in the coastal and selva areas.

In a recent survey of Heterodera rostochiensis in Peru the mountainous agricultural regions, but not the coastal valleys, were found to be generally infested. This distribution was confirmed in the present survey, with the exception that cysts of this nematode were found in 14 of the 37 soil samples collected in the Huaras area. No cysts had been reported previously from that area. H. rostochiensis is believed to be endemic to Peru (25).

The genera Pratylenchus, Tylenchorhynchus, Rotylenchus, Helicotylenchus, and Trichodorus were widespread in all three areas. Species of these five genera were particularly numerous in the mountains, whereas fewer species and individuals were encountered in coastal and selva areas. An exception was Helicotylenchus erythrinae which was present, mostly in large numbers, in 54 of the 61 samples collected in the selva and was the most frequent nematode of all species of this area.

Tylenchorhynchus capitatus was frequently encountered in large numbers in association with small grains in the mountains of Peru. Recently, this species has been recovered repeatedly from tobacco plant beds in the mountainous region of North Carolina. Host range tests in the greenhouse by the junior author proved its pathogenicity and large populations were obtained on Burley tobacco, tomato, wheat, oats, cotton and corn.

Xiphinema was the most frequent genus in the coastal area and was also abundant in soil samples from the selva.

Species of the genera Criconemoides and Hemicriconemoides were found mostly in small populations in soil samples from the mountains and selva.

Paratylenchus, Hemicycliophora, and Hoplolaimus seem to be of the least importance since they occurred only in small numbers in a few samples.

Tylenchulus semipenetrans may become quite important on citrus in the selva.

Most of the nematodes found in association with various crops in Peru are world-wide in distribution. In addition, however, several species found were described in recent years and are known only from a few localities. Such species include Pratylenchus thornei, P. minyus, Tylenchorhynchus capitatus, T. martini, T. brevidens, Rotylenchus boocki, Trichodorus christiei and T. monohystera. The fairly common occurrence of these nematodes in Peru suggests that they may have a much wider distribution than was previously indicated.

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REPORT ON 1957 FIELD TESTS OF ACTI-DIONE DERIVATIVES FOR CONTROL OF BLACK STEM RUST OF WHEAT[1]

R. G. Hacker and J. R. Vaughn[2]

Summary

Further field tests of Acti-dione "S" (the semicarbazone of the cycloheximide) for control of black stem rust of wheat confirm that this compound is extremely effective when used at high concentrations and rates. The tests, conducted at the Wyoming Agricultural Experiment Station, resulted in excellent control of rust, with mean yields as much as 60 percent higher than non-treated checks. The degree of control is related directly to the amount of material applied.

In 1956, tests with new derivatives of Acti-dione[3] (semicarbazone, acetate, and oxime of cycloheximide) resulted in outstanding control of Puccinia graminis tritici on the spring wheat variety Onas (1, 2). The semicarbazone compound produced long-term "resistance" resembling disease immunity set up in animals by a vaccine. Applied twice, at late-boot and middle-flowering stages, at a concentration of 200 ppm (200 gallons and 50 gallons per acre respectively) this material furnished complete protection against rust infection for the balance of the growth and maturation periods. The sister compounds, the acetate and oxime, were partially effective.

Since the mode of action of these chemicals is unknown, the term "induced chemical resistance" was used to describe their end effect. Whether the phenomenon was true induced resistance through alteration of the plant metabolism, or simple systemic chemical protection, treated plants resisted infection by rust for the remaining 45 days of their developmental cycle.

Tests of these same materials were conducted during the past, 1957, season at the Wyoming Station to expand and reproduce, if possible, the 1956 results.

PROCEDURE

All physical conditions of the 1957 experiment were held as closely as possible to those of the 1956 tests. Replicated rod-row plots of Onas wheat were planted May 15 on an irrigated block adjacent to that used in 1956. First treatments were applied June 14 to 17 (as compared with July 23 in 1956) when the average growth stage of the plots had reached late boot. These consisted of the semicarbazone, acetate, and oxime derivatives in varying formulations, concentrations and rates.

In the balance of this report these compounds will be referred to by their new designations: Acti-dione "S", "A", and "O", respectively, instead of semicarbazone, acetate, and oxime.

Control of Special Factors

One of the primary objectives of these tests was to duplicate the exact conditions under which Acti-dione "S" yielded the spectacular results referred to above. Since other workers had used this material without similar success, the Wyoming procedure was analyzed for differences in technique which might account for this. Three main variables, excluding climatic factors and other conditions not controllable, appeared to be present in our method: 1) concentration and rate of material applied, 2) time of application, and 3) formulation of solutions. Our methods were as follows:

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[1] Published with the approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 107.

[2] Graduate Research Assistant and Assistant Director, Wyoming Agricultural Experiment Station respectively, University of Wyoming.

[3] Registered trade name, The Upjohn Company.

Table 1. Summary of treatments, yields and rust readings.

Treatment No.	Material and formulation	Concentration (ppm)	Rate gallons per acre	Mean Yield bushels per acre	Percent of check	Rust readings Average condition: Soft dough stage: Stem rust percent[a]	Soft dough stage: Stripe rust[b]	At maturity: Stem rust percent	At maturity: Stripe rust
1	Crystalline "S" plus Dynawet (2 applications)	200 200	200 50	66.7	160**	5	Light	15	Moderate.
2	Crystalline "S" plus Dynawet (1 application)	200	200	54.7	132**	10	Moderate	25	Moderate-severe
3	Crystalline "S" plus Dynawet (1 application)	200	50	45.6	110	10	Moderate	40	Severe
4	Upjohn Lab. Formulation "S"-67 (1 application)	150	30	47.5	115	30	Severe	50	100%
5	Non-treated check	--	--	41.4	--	25	Severe	75	100%

**Yield increases for Treatments 1 and 2 highly significant at .01 level. Others n. s.

[a]Stem rust readings in percent, Cobb Scale

[b]Stripe rust estimated on basis of: Zero, Light, Moderate, Severe, 100%.

1. Concentration and Rate: Greenhouse tests had indicated that 200 ppm of Acti-dione "S" and "A" in aqueous solution were effective and below the phytotoxic level when plants were sprayed until "thoroughly wet". In the field, thorough wetting (to run-off) dictated a rate approaching 200 gallons per acre -- not a practical volume for large-scale application, but one which had to be tested in the light of the preliminary indications.

2. Time of Application: In the 1956 field tests, solutions of the "S" and the "A" compounds had been applied in the evening, after dark, when dew had formed on the plants. Leaves of the plants were literally bathed in the antibiotic solutions until drying occurred the following day. This was a period of approximately 9 hours. It should be noted here that both Acti-dione "S" and "A" are almost totally insoluble in water, and after they recrystallize from a stable formulation, they are not resolvable. The evidence indicates that the period of time that the solution is available for absorption by the plant is a significant factor in uptake.

3. Formulation of Solutions: The pure crystalline compounds were put into stable solution through the following solvent series: 1) dimethyl sulfoxide (minimal volume); 2) 95% alcohol to equal 1.0 percent of the total field volume; 3) tap water to full volume.

Dow Dynawet was added to each tank mix at a rate of 1:1000.

Variations from 1956 Procedure

Certain uncontrolled variables entered into the 1957 tests which were not present in the original experiment. These were:

1. Early Spore Showers: Normally, rust-spore showers do not occur in the Laramie area until late July or early August. Last summer there were showers of both Puccinia

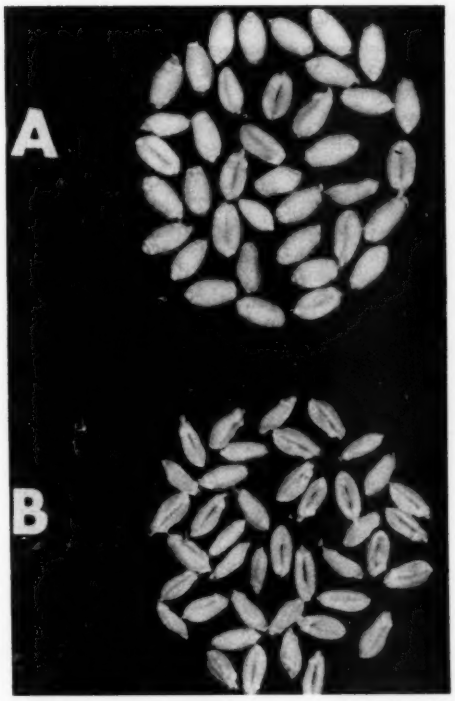

FIGURE 1. Representative grain samples from: (A) Treatment No. 1 -- Acti-dione "S", 200 ppm, 2 applications; (B) Non-treated check plots.

FIGURE 2. Check plot, nearing hard dough stage: stem rust 65 percent; stripe rust 100 percent (leaves completely flagged).

graminis tritici and P. glumarum which caused primary infection just before the time that pre-infection treatments were made. The Acti-dione derivatives are ineffective against established infections but seem to prevent secondary build-up of stem rust.

2. Rust Epiphytotic: Both stem rust and stripe rust became epiphytotic before the grain reached full maturity. The inoculum potential was much higher than in 1956. (In 1956 only moderately severe stem rust occurred and then only late in the season after heads had reached the hard dough stage.)

3. Abnormally Wet Season: During the early growth stages and continuing into the flowering stage, heavy rainfall kept the plots saturated. These conditions favored succulent growth and high susceptibility to the early rust invasions.

4. Powdery Mildew: Moisture conditions similarly favored development of powdery mildew (Erysiphe graminis tritici) on the lower stems and leaves. The mildew was moderately severe at the time applications were made but decreased in severity thereafter.

RESULTS

Four of the treatments using Acti-dione "S" effected control of rust varying in degree from slight to excellent and produced corresponding increases in yield (see Table 1). No

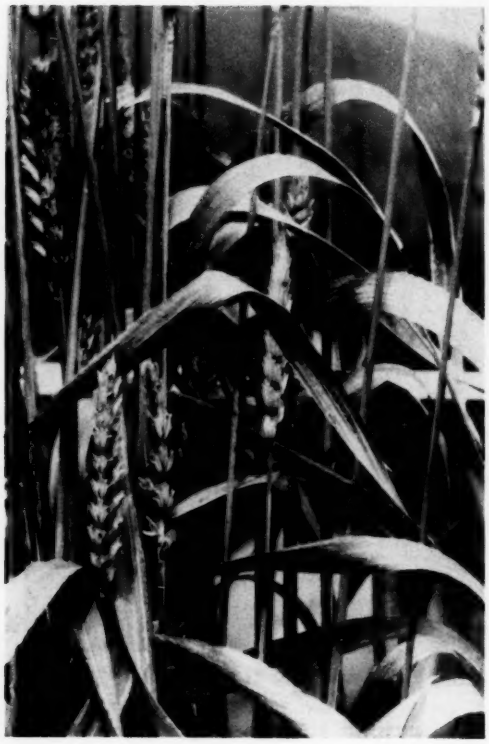

FIGURE 3. Photograph of treated plot No. 1, taken at same time as Figure 2. Stems relatively free of stem rust; stripe rust moderate; leaves still relatively green and functional.

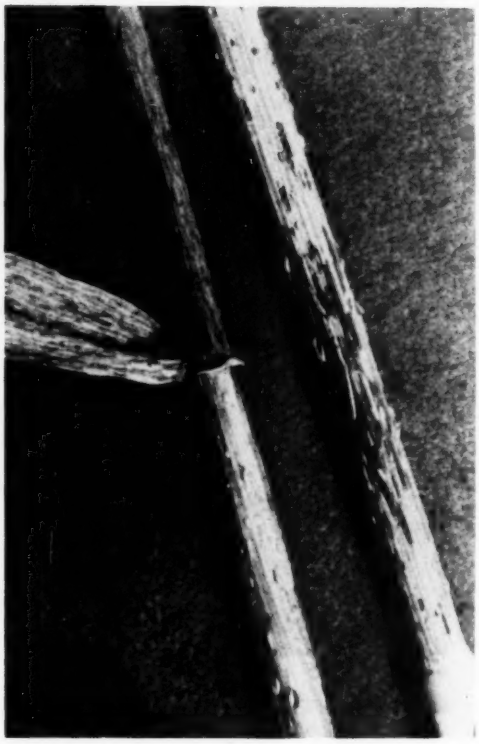

FIGURE 4. Stems from non-treated check plots severely infected with stem rust.

other treatments resulted in disease control.

Plots receiving treatment No. 1 (crystalline "S" with Dynawet, two applications) had a mean yield of 66.7 bushels per acre, an increase of 60 percent over the yield of 41.4 bushels from non-treated check plots. Near maturity these plots showed stem rust averaging only 15 percent severity, and stripe rust rated as moderate (Fig. 1 - 4).

Treatment No. 2 (crystalline "S" with Dynawet, 1 application) resulted in a mean yield of 54.7 bushels per acre, or 32 percent more than the checks. Stem rust averaged 25 percent and stripe rust was moderately severe.

Treatments 3 and 4, in which rates of application were much lower, reduced rust only slightly. The apparent increases in yield were not significant statistically.

All other treated plots showed rust infection equal in severity to the non-treated checks.

Since the date that first pustules appeared indicated that infection had occurred before treatment, this is further evidence that Acti-dione "S" will not eradicate existing infections but does inhibit or prevent new infections. Referring to Table 1, it should be noted that the degree of inhibition throughout the maturation period was higher for treatment No. 1 than for No. 2, which indicates a direct relationship to the total amount of chemical applied.

CONCLUSIONS

1. Acti-dione "S", applied as a pre-infection treatment at a sufficiently high concentration and rate, effects excellent control of stem rust.
2. The degree of control increases directly with the amount of material applied.
3. Time of application and the period of time that the antibiotic solution remains available to the plant are factors which appear to be important but as yet unproved.
4. The effective rate and dosage indicated are not economical. Further research is needed to find ways of reducing these and enhancing uptake by the plant.

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WYOMING AGRICULTURAL EXPERIMENT STATION

REACTION OF SPRING AND WINTER BARLEYS TO CULTURES OF FOUR RACES AND CULTURE P35 OF USTILAGO NUDA

J. G. Moseman and David A. Reid[1]

Summary

The four standard differential varieties used by Tapke (4) had the same reaction to infection with culture 269 of race 1 and culture P35. However, the Ogalitsu variety (C.I. 7152[2]) was resistant to culture 269 and susceptible to culture P35. Therefore, culture P35 can be considered a biotype of race 1. Eleven of 13 spring barley varieties and selections and 4 of 19 winter barley varieties and selections tested were found to be resistant or immune to infection with cultures of races 1, 2, 3, and 4 and culture P35.

INTRODUCTION

Loose smut, Ustilago nuda (Jens.) Rostr., is one of the most serious diseases of barley in the United States. In some States, certain varieties of barley have not been recommended by the State Experiment Stations chiefly because of their susceptibility to this disease. In most States there is little or no tolerance of loose smut permitted in fields accepted for certification.

In recent years the methods for treating seed to kill the loose smut fungus inside the kernel have been greatly improved. Some of these methods for seed treatment can be done on the farm but they require the soaking of the seed and thus are not used very extensively. The most desirable method of controlling loose smut is still by the growing of resistant varieties.

In this study, varieties found by various investigators to have resistance to loose smut were inoculated either with a composite of or with individual cultures of each of the four physiologic races reported by Tapke (4), and with culture P35 from Cloninger and Poehlman (1).

MATERIALS AND METHODS

The seed of the selections from the cross of (Vantage x Jet) x Vantmore were furnished by W. H. Johnston, Brandon, Manitoba. The four Georgia selections were furnished by A. R. Brown, Athens, Georgia. They originated from lines that were distributed from J. W. Taylor's breeding program at Beltsville, Maryland, in 1951. Many of the remaining varieties and selections were furnished from the World Collection of Barleys by D. J. Ward, Beltsville, Maryland. The authors wish to express their appreciation to these individuals for furnishing this seed.

The four cultures, one of each of the four physiologic races described by Tapke (4), had been used in his studies of U. nuda. The origin of these cultures was as follows: culture 269 of race 1 from North Dakota; culture 244 of race 2 from South Dakota; culture 183 of race 3 from Minnesota; and culture 267 of race 4 from Kansas. The culture P35 was furnished by J. M. Poehlman, and was originally obtained from Kinroku Selection Mo. B467 in the barley breeding nursery at Columbia, Missouri. The culture P35 was found by Cloninger and Poehlman (1) to be virulent on many varieties and selections which they and other workers were using in their programs to develop winter barleys resistant to loose smut. The inoculum in the composite consisted of approximately equal amounts of chlamydospores of each of the cultures of the four races.

The present study was conducted during the winter months in the greenhouse at Beltsville, Maryland. Since cultures of the pathogen were used that were not present in this area, inoculations were made and infected plants were grown when barley growing in the field was in the stage of development in which it could not become infected.

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[2] C.I. refers to the accession number of the Cereal Crops Research Branch, Crops Research Division.

Table 1. Reaction of five varieties of barley when inoculated with cultures of four physiologic races and culture P35 of Ustilago nuda.

Culture No.	Physiologic race	Reaction of variety: Charlotte-town	Compana	Valki	Golden Melon	Ogalitsu
269	1	S	R	R	S	R
244	2	S	S	S	S	R
183	3	S	S	R	S	R
267	4	R	R	R	S	R
P35	1A	S	R	R	S	S

a Reaction: S = more than 10 percent of plants smutted, R = 10 percent or less of plants smutted.

Table 2. Reaction of 13 spring barley varieties and selections when inoculated with five cultures of Ustilago nuda.

Variety or selection	C.I. No.	Reaction[a] to culture: P35	R1	R2	R3	R4	Composite[b]
Jet	967	I	I	I	I	I	
Anoidium	7269	R	I	I	I	I	
(Vantage x Jet) x Vantmore, Br. 5479-690[c]	--	I	I	I	I	I	
" " " -675	--	I	I	I	I	I	
" " " -755	--	I	I	I	I	I	
" " " -680	--	I	I	I	I	I	
Trebi	936	I					S
Abyssinian	668	I					I
Bifarb	3951-3	-					I
Kitchin	1296	I					R
--	3694	R					I
--	5324[d]	I					I
--	5798	I					I

a I = 0 percent plants smutted, R = 10 percent or less plants smutted, S = more than 10 percent plants smutted.

b Composite = a composite of the cultures of races 1, 2, 3, and 4.

c Selections from this cross of (Vantage x Jet) x Vantmore are now being referred to as "Meteor lines".

d R. G. Shands, Madison, Wisconsin, unpublished data, found C.I. 5324 to be susceptible to a collection of U. nuda from Wisconsin which is pathogenic on Trebi.

Table 3. Reaction of 19 winter barley varieties and selections when inoculated with 5 cultures of Ustilago nuda.

Variety or selection	C. I. No.	Reaction[a] to culture					
		P35	R1	R2	R3	R4	Composite[b]
Wong	6728	S	S	S	S	S	S
--	4966	I	I	I	I	I	
--	3210-5	I					I
Clancy	1002	S					S
Hooded 16	6574	S					S
Kenbar	7574	S					S
North Carolina 26	7026	S					R
M.E.B. Sel. Mo. B404	10046	S					S
" B405	10047						S
" B411	10048	S					I
" B472	10052						S
" B502	10054						S
" B507	10055						S
" B538	10056						S
" B539	10057						S
(Hooded 16 x Sunrise) Tenwase x Wong-Jet, Ga. Sel. 1034	--	I					I
Ga. Sel. 1064	--	S					I
N. C. 23-Def-Sunrise x Valki-Wong Ga. Sel. 1111	--						S
(Hooded 16 x Sunrise) Tenwase x Wong-Jet, Ga. Sel. 1134)	--	I					I

[a] I = 0 percent plants smutted, R = 10 percent or less plants smutted, S = more than 10 percent plants smutted.
[b] Composite = a composite of cultures of races 1, 2, 3, and 4.

The florets were inoculated with dry spores on the day of pollination, or the day before or day after pollination. In 1955 and 1956 a sharp pointed forceps was used to deposit the chlamydospores of the loose smut fungus on the stigma of each individual floret by the method described by Tapke (4). In 1957 the flowers were inoculated with a hypodermic needle to which was attached a small rubber bulb containing dry spores, as described by Shands and Schaller (2).

Inoculated seeds of the winter varieties were vernalized by germinating the seeds on moistened blotters in petri plates for 36 to 48 hours, or until the coleoptile had just emerged. The seeds were then refrigerated at 33 to 34° F for 3 to 4 weeks in moist sand in 4- by 8-inch tin pans, 2 inches deep. At the end of this period the coleoptiles were usually less than an inch long. The vernalization was started so that the seedlings could be transplanted about September 10 to 15 when maximum greenhouse temperatures were not excessive. After vernalization, the pans were kept at room temperatures for 24 to 48 hours or until the coleoptiles were 1 to 2 inches long. The seedlings were then transplanted 1 inch apart in rows 3

or 4 inches apart in a greenhouse bench, similar to the method described by Tapke (3). Inoculated seed of spring varieties was planted directly in the greenhouse bench. Supplementary mazda lights were added from 12 midnight to 2 a.m. on both winter and spring varieties, beginning when the seedlings were in the three- to four- leaf stage. Data were recorded in percentage of plants infected, and were obtained on an average of 20 plants of each variety or selection for each culture.

RESULTS AND DISCUSSION

The four varieties that Tapke (4) used to differentiate races, plus the Ogalitsu variety (C. I. 7152), were inoculated with a culture of each of the four physiologic races that he identified, and with culture P35. The reaction of these five varieties when inoculated with the five cultures is shown in Table 1. Reaction of four differential varieties to the cultures of races 1, 2, 3, and 4 was the same as was found by Tapke, and to culture P35 the same as to culture 269 of race 1. However, Ogalitsu was resistant to culture 269 of race 1 but susceptible to culture P35. This indicates that culture P35 differs from culture 269 in pathogenicity and should be considered a biotype of that culture.

The reaction of 13 varieties and selections of spring barley when inoculated with the five cultures is shown in Table 2. These varieties and selections were tested because they were previously reported to be resistant to some cultures of loose smut and are being used in breeding to develop varieties resistant to loose smut. All of the varieties and selections included in this table except Trebi were either immune or resistant to infection with all the cultures with which they were inoculated. Trebi was immune to infection with P35 but was susceptible to infection with the composite of the other four cultures. Trebi has been reported by Shands and Schaller (2), Tapke (4), and others to be susceptible to some cultures of loose smut. Shands [3] found C. I. 5324 to be susceptble to a collection of U. nuda from Wisconsin which is pathogenic on Trebi.

The reaction of 19 winter barley varieties and selections when inoculated with five cultures of loose smut is shown in Table 3. Only four of the varieties or selections were immune to infection by all five cultures. The variety C. I. 4966 had been shown previously by Cloninger and Pehlman (1) to be immune to culture P35. The variety C. I. 3210-5 may offer a new source of resistance to loose smut. The two selections from Georgia probably acquired their resistance from Jet, and although they are not a new source of resistance, they are a source of the Jet resistance in a winter variety. The 14 varieties that were found to be susceptible to either culture P35 or a composite of the other four cultures had previously been reported by Tapke (4) to be resistant to all the cultures with which he had inoculated them.

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[3] R. G. Shands, Madison, Wisconsin, unpublished data.

THE SALT-WATER SOAK TREATMENT FOR THE CONTROL OF LOOSE SMUT OF BARLEY[1]

R. C. Russell and S. H. F. Chinn[2]

Summary

This paper reports results of field experiments in controlling loose smut of barley (caused by Ustilago nuda (Jens.) Rostr.) by soaking the infected seed in weak saline solutions. This treatment, compared with the water soak treatment, was just as effective in controlling the disease and interfered much less with germination of the seed.

INTRODUCTION

During recent years several variations of the water soak treatment, for the control of loose smut of barley, have been developed. Tyner (7, 8) at first used Spergon in the water in which the seed was treated, but later found that the disease could be controlled by the use of water alone. Russell (5) also demonstrated that soaking the infected seed in water resulted in the control of this disease. Leben and Arny (4) confirmed the fact that both the Spergon treatment and the water soak treatment were effective for this purpose. Russell (6) and Hebert (3) both emphasized the value of excluding oxygen from the seed during treatment. After soaking the seed for a few hours, Hebert drained off the water before completing the treatment in an air-tight container. Chinn and Russell (1) secured control of the disease by soaking the seed in a broth culture of an antibiotic bacterium, but at the same time found that the same measure of control could be attained by soaking the seed in water for a somewhat longer period. In later work (2) they obtained better results through the use of salt (NaCl) in the water in which the seed was treated.

The purpose of the present paper is to report the results obtained in the field during the past season with the salt-water soak treatment.

MATERIALS AND METHODS

Of several experiments conducted with this treatment in field plots, only two are dealt with in detail because the results of these are typical of those obtained in all of the tests. In both of the experiments described below, naturally infected seed was employed, and the varieties chosen were, for the most part, ones that were in general use in Western Canada.

In the first experiment seed of three varieties was soaked in water or in 1% or 2% aqueous solutions of common salt for 58 hours at 76° F. After treatment the seed was air-dried for several days and then sown in plots consisting of five 8-foot rows, replicated five times. These plots were randomized. The results of the experiment are shown in Table 1.

These data show that the salt-water soak treatment gave essentially the same measure of control as did the water soak treatment. In addition the salt-water soak resulted in considerably better yields. The 1% solution gave slightly better results than the 2% solution.

This experiment illustrates very clearly the great differences that occur in the reaction of different lots of seed to the soak treatments. The Titan seed was severely damaged and the resultant crop produced a very low yield. On the other hand, the Husky sample suffered very little damage and even the water soak plots yielded quite well. The Montcalm seed was intermediate in this respect.

In the second experiment the efficiency of the two treatments was compared and the influence of the length of treatment was studied. The seed of four varieties was subjected to the different treatments for periods of 55, 65, or 75 hours, at a temperature of approximately 72° F. In this experiment ten 12-foot rows of each variety were devoted to each treatment. The results of this experiment appear in Table 2.

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[1] Joint contribution from the Botany and Plant Pathology Division (No. 1675) and the Bacteriology Division (No. 456) Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Plant Pathologist and Bacteriologist, respectively. Plant Pathology Section, Canada Department of Agriculture Research Laboratory, University Sub Post Office, Saskatoon, Saskatchewan.

Table 1. A comparison of the water soak treatment and two variations of the salt-water soak treatment in controlling loose smut of barley.

Variety	Treatment	Total No. of heads		Yield *
		Healthy	Smutted	
Husky	Water soak	3393	3	91
	Salt-water soak 1%	4233	0	113
	Salt-water soak 2%	4190	3	112
	Untreated check	3741	383	100
Montcalm	Water soak	1748	0	55
	Salt-water soak 1%	3371	0	106
	Salt-water soak 2%	3203	1	101
	Untreated check	3169	192	100
Titan	Water soak	1127	0	33
	Salt-water soak 1%	2916	2	85
	Salt-water soak 2%	2694	6	79
	Untreated check	3420	260	100

* The "yield" in this case is the number of healthy heads expressed as a percentage of the number of healthy heads in the corresponding check

The degree of control achieved was essentially the same whether water or salt solutions were used. In the matter of yields, however, the salt-water soak treatments were decidedly superior to the water soak treatment. With each increase in the length of the soak the water soak treatment caused increased injury to the seed. Except for plots containing the variety Rex, the plots sown with seed receiving the salt-water treatments usually outyielded the untreated checks.

The length of treatment required to give complete control of the disease, at 72° F, was 75 hours for the Gateway, Rex, and Husky samples, but 65 hours was sufficient to eliminate the smut from the Montcalm barley. Even the 55-hour treatment gave fairly good control in some instances. Lengthening the water soak treatment decreased the yields quite noticeably, but this tendency was not so marked with the salt-water treatments.

DISCUSSION

For some years now, several soak treatments have been known that would give satisfactory control of loose smut of barley, if properly applied. The authors have tested most of these newer treatments and the hot water treatment as well. In their experience all of these treatments interfered seriously with the germination of certain samples of seed. Certain other samples survived the treatments with little or no loss in germination.

The salt-water soak treatment appears to have an advantage over all the other treatments tested because it interferes less with the germination of the seed. The results of our comparative germination tests are reported in a separate paper recently submitted for publication.

The addition of salt to the water in which the seed was treated has frequently led to increases in the resultant yields when compared with those of the untreated checks.

The salt-water soak treatment is somewhat cumbersome, but it is relatively inexpensive. It is felt that, with a relatively small outlay of time and energy, most seed-growers could treat enough seed every year to sow a seed plot large enough to produce all their seed requirements for the following year.

Table 2. The relative efficiency of variations of the soak treatment for the control of loose smut of barley.

Variety	Length of soak in hours	Concentration of salt in %	Percentage of heads smutted	Yield in % of the yield of checks
Gateway	55	nil	0.2	96
	55	1	0.1	108
	55	2	0.5	102
	65	nil	0.3	99
	65	1	0.1	108
	65	2	0.3	104
	75	nil	0	86
	75	1	0	110
	75	2	0	105
	check	untreated	4.8	100
Rex	55	nil	0.5	42
	55	1	1.8	70
	55	2	0.8	85
	65	nil	0	23
	65	1	0.3	63
	65	2	0.6	70
	75	nil	0	11
	75	1	0	56
	75	2	0	97
	check	untreated	5.7	100
Montcalm	55	nil	0.3	94
	55	1	0.2	108
	55	2	0.1	109
	65	nil	0	62
	65	1	0	100
	65	2	0	96
	75	nil	0	4
	75	1	0	107
	75	2	0	118
	check	untreated	3.5	100
Husky	55	nil	1.9	106
	55	1	0.2	108
	55	2	2.2	102
	65	nil	0.1	95
	65	1	0	102
	65	2	0.3	103
	75	nil	0	89
	75	1	0	127
	75	2	0	116
	check	untreated	17.6	100

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PYRENOCHAETA TERRESTRIS AND PHAEOCYTOSPORELLA ZEAE ON CORN ROOTS [1]

Jeweus Craig and Benjamin Koehler

Pyrenochaeta terrestris (Hansen) Gorenz, Walker, and Larson and Phaeocytosporella zeae Stout were among the eight species of fungi (Fig. 1) tested for their ability to cause the red root rot condition of dent corn (Zea mays indentata L.). The organisms tested were selected from a group of fungi isolated from roots of field corn showing red rot in early fall on the Agronomy South Farm of the University of Illinois.

Six species of Fusarium were isolated, but Fusarium graminearum Schw. (Gibberella zeae (Schw.) Petch), a very common corn stalk rot pathogen in Illinois, and sometimes reported in the literature as a root pathogen of corn was not among the fungi isolated, nor was Diplodia zeae (Schw.) Lév. found.

The red rot condition has been ascribed to Pyrenochaeta terrestris by some investigators (1, 3, 7). Other organisms have also been named as the causal agent (2, 6, 7). P. terrestris also has been reported as causing lesions on the basal areas of corn stalks (4).

Phaeocytosporella zeae has been reported as a cause of or at least associated with stalk rot in the United States and France (4, 5, 8). Its ability to invade corn roots previously has not been reported.

In this investigation seed of the corn hybrid (Ind. WF9 x CI. 187-2) (Ill. Hy2 x Ia. L317) was planted in pots of soil which had been steam-sterilized and then artificially infested with the fungus being tested, as follows: The infested soil was placed in the bottom part of the pot and covered with a 1/2-inch layer of sterile soil on which the seed was placed and covered with sterile soil. The seedlings were grown in these pots at temperatures of 15°, 25°, 27°, and 37°C for periods of 9, 5, 4, and 2 weeks, respectively. At the conclusion of the experiment, the seedling roots were washed free of soil, weighed, and examined for damage. All dark discoloration or necrosis was considered as damage. Isolations were made to determine the species of fungi present in the tissues.

Pyrenochaeta terrestris was the only species among the eight isolates tested that induced a red rot of corn seedling roots. The color of the rot ranged from pink to dark red. Temperature affected both the intensity of color and the extent of the rot. Corn seedlings grown in soil infested with P. terrestris at a temperature of 25°C exhibited a deeper red and more extensive rot than those at the lower and higher temperatures.

Four isolates of P. terrestris were used in this investigation. These isolates, from corn roots collected at four different locations, differed appreciably in their damage to seedling roots. The various isolates of P. terrestris and Phaeocytosporella zeae are designated by numbers in Figure 1.

Root invasion by P. terrestris was confined primarily to the cortex with a lesser invasion of the stele. Seminal roots had the greatest amount of rot and damage to adventitious roots increased in direct proportion to their age. The frequent isolation by the writers of P. terrestris from corn roots showing red rot in the field, and its ability to induce this rot in corn seedlings under experimental conditions, indicate it is the primary cause of red root rot. These conclusions agree with those of Johann (3).

Phaeocytosporella zeae and Pyrenochaeta terrestris were the only organisms among those tested that caused extensive and consistent rot of the corn seedling roots (Fig. 1). P. zeae caused a dark brown rot of the roots with hyphal invasion predominantly confined to the cortex. As with P. terrestris the seminal and older adventitious roots showed the greatest damage. Root damage from P. zeae was the same at temperatures of 15°, 25° and 27° C, but the fungus was not found to be present in root tissues of seedlings grown at 37°.

Differences in root damage ratings attributable to the various fungi used (Fig. 1) were significant at the 1% level at 15° as well as at 25° C. The most noticeable above-ground symptom due to inoculation was exhibited by the seedlings grown at 15° in soil infested with Penicillium oxalicum. Forty percent of these seedlings died as compared to a 4 percent average loss in the other treatments at the same temperature.

The extent of the significance of Pyrenochaeta terrestris and Phaeocytosporella zeae as parasites of corn roots is not yet clear. There was no doubt about their ability to cause root damage of young plants under the conditions of this experiment, but the fresh top and root weights were not significantly different from those of the check.

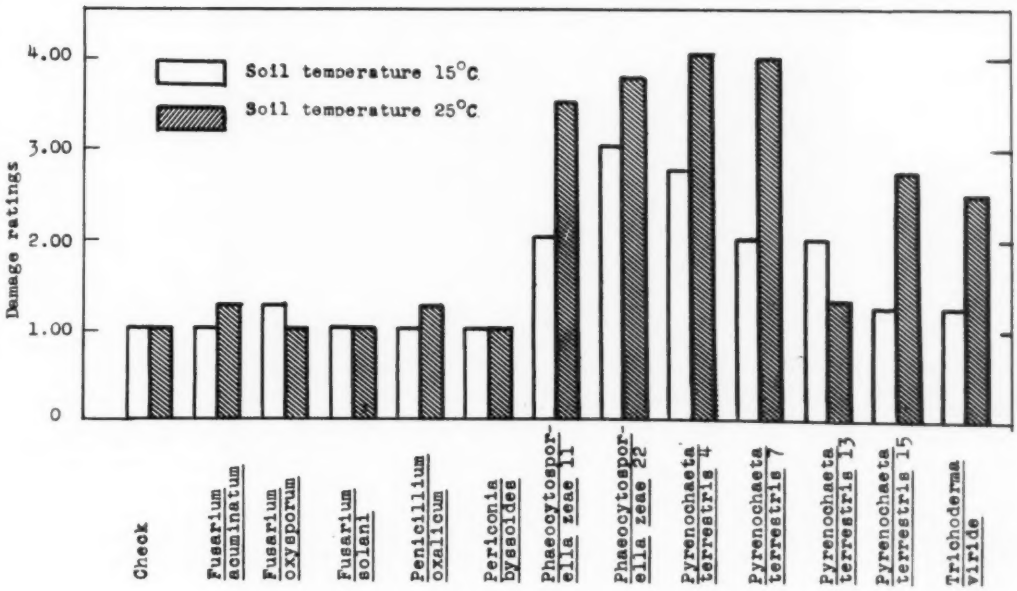

FIGURE 1. Comparisons of the amounts of damage by the fungi tested to the roots of corn seedlings grown at temperatures of 15° and 25°C. System of rating: 1.00, damage slight; 2.00, slight to 10 percent; 3.00, 11 to 25 percent; 4.00, 26 to 40 percent, of the root system.

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OBSERVATIONS ON FUNGUS DISEASES OF RICE IN FLORIDA 1951-1957[1]

Victor E. Green, Jr.[2]

Summary

Disease observations in connection with rice production in the Everglades area of Florida during the 7-year period 1951-1957 are reported. Brown spot, narrow brown leaf spot, Rhizoctonia sheath spot, and seedling blight were observed regularly, but did not seriously affect rice production. The only serious rice disease was leaf and neck blast, which occurred in epiphytotic proportions each year. Measures to overcome the effects of the disease are discussed.

DISEASES RECORDED

Work on rice culture in the Everglades began early in 1951, when rice growers from Louisiana and Texas moved to that area seeking level land with an abundance of fresh water. Production of rice was not agronomically practical on those organic soils until ferrous sulfate was added to correct a physiological disease resulting in "straighthead", or empty glumes (5). In 1951 a 160-acre planting of rice was abandoned and replanted in August. The crop matured in December, having grown disease-free, with the exception of a light infection of brown spot caused by Cochliobolus miyabeanus (Ito & Kuribayashi) Drechsler ex Dastur = Helminthosporium oryzae Breda de Haan. All experimental and commercial plantings here have been observed to be infected with brown spot, but there is no indication that there has been a reduction in vigor or yield due to that disease (Figure 1).

Symptoms resembling Rhizoctonia sheath spot, caused by Rhizoctonia oryzae Ryker & Gooch, were observed on individual plants in commercial fields each year, but there seemed to be little damage to the rice plants outside the lesions, which numbered only a few per plant.

Narrow brown leaf spot, caused by Cercospora oryzae Miyake, occurred each year in commercial and experimental plantings, but since it appeared to be of no consequence in the area no attempt was made to identify the races or to check varietal response. The extreme lodging observed in fields of Zenith rice were not attributable to infections by Cercospora.

One case of seedling blight, in epiphytotic proportions, caused by Corticium rolfsii Curzi = Sclerotium rolfsii Sacc., occurred in a commercial field of rice following celery in which almost one-third of the celery plant yield was disked into the soil. Rice was seeded immediately. About a week after emergence the rice plants began to wither, and about 10 percent of them died. An early irrigation stopped the progress of the disease, and the remaining plants were saved. This disease is not easily distinguishable from wireworm (Melanotus communis Gyll.) injury, which may occur simultaneously with seedling blight. However the "pseudo-albino" condition, wherein the leaves contain white stripes or the entire leaf becomes white, is readily distinguishable from hoja blanca disease and from the nutritional disorder reported by Green and Stoner (1952).

BLAST DISEASE

The only fungus disease that presented a serious problem to rice culture was that caused by Piricularia oryzae Cav. Plants attacked in the seedling stage suffered major damage in that considerable active leaf surface was lost. Many plants were killed. The loss of stand resulted in greater stooling by plants that survived, but in some cases increased weed growth more than offset the effect of stooling. In cases of head blast, or "rotten neck", infections occurred at various points on the panicle, either singly or in combination. At times only the pedicel supporting an individual spikelet was infected. In other cases a branch of the rachis broke over, blasting the numerous spikelets on a branch. A number of the branches might be

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FIGURE 1. Mature panicle of Zenith Rice showing damage by lesions of brown spot. The grains were not affected, but the glumes had an unsightly appearance.

similarly affected. In extreme cases, when a lesion girdled the main rachis of the panicle just above the flag leaf, none of the spikelets filled with grain.

Infection occurred both before and after the grain formed. In the former case the spikelets did not fill. In the latter case the effect on yield was through shattering brought about by wind breaking off pedicels, branches, and rachillae that could not be recovered during harvest.

In commercial plantings a 10-day difference in planting of the same variety meant the difference between early neck blast, with subsequent crop failure, and late neck blast, after the spikelets had formed grain. Even in cases of late neck blast, the secondary tillers suffered since grain did not form in the panicles.

During the crop years 1952 to 1957, the U.S.D.A. world collection of rice varieties was grown six times[3]. At Belle Glade the nursery was used chiefly to test the susceptibility of up to 2200 varieties of rice to blast disease. Green and Tullis (3) reported that no correlation was shown between length of growing period or geographical origin of varieties and susceptibility to blast disease in Florida. Late February or early July seedings were suggested as possible aids in maturing plants early or late enough to escape blast in susceptible varieties. The use of balanced fertilizers will keep the plants less susceptible to blast. For commercial production, the medium grain variety Lacrosse or the short-grain variety Caloro were recommended for planting in south Florida at a seeding rate of 75 pounds per acre. Bluebonnet 50 and Century 52 were more resistant to blast disease than Bluebonnet and Century 231, respectively. Unreleased varieties, relatively free of leaf spot diseases during the 1953 season, were SM BR-BR41 x CI 8326, B.R. x (Rexark-B.R.), C-3-1-9-2-4, and C-6-1-25-12 B.R. x B.R.-R.

During the 1954 crop year Green and Schein (2) determined that the following varieties in the U.S.D.A. Uniform Nursery were resistant to blast: LaCrosse x Magnolia, 250-3 x Magnolia, Hill Selection x Bluebonnet, Asahi, and Calrose. Of 75 United States rice varieties, including 51 unreleased varieties, 30 were resistant.

Studies made in 1955 and 1956, reported by Green and Van Arsdel (4), showed that periods of extensive blast disease spread occurred when minimum night temperatures were warm. In 1955, on muck soils, late July, August, and early September were most favorable to blast spread. Young rice that had tillered was most susceptible. Head blast spread rapidly only in periods when night temperatures were above 72°F. Flooding rice in the pretillering stage in July and August caused a local warming of the air over the paddy of 4° to 5°. Thus a flooded field of young rice had more favorable temperature for blast than unflooded rice. Cloudy weather with assorted warm nights favored spread of the disease. Laboratory studies and specific night inoculations indicate that infection rarely occurred at 68° or less. Sunlight was detrimental to spore viability and infection. In 1955 rice planted April 27 largely escaped infection, while May 2 plantings had only small quantities of artificially induced blast. The April 1 and May 1, 1956, plantings escaped leaf blast.

Green and Panzer (1) made drilled plantings of three commercial varieties (Zenith, Century 231, and Rexoro) at monthly intervals in April, May, June and July of 1956. One series was irrigated while another was kept under dry land culture. Blast intensity was related to weather conditions. The disease was not observed in the April planting, but was found to be mild, severe, and very severe in the May, June, and July plantings, respectively. Reduction in yield due to head blast was 0, 25, 100, and 100 percent, respectively, for the four planting dates. The same trend was observed in both irrigated and non-irrigated rice, although slightly more blast was found with dry land culture. Occurrence of blast was related to periods of rainy weather during warm nights, when the minimum night temperatures were in the high 60's or above. Zenith was found to be the least susceptible of the varieties tested. Early planting, in March or April, was again suggested as a cultural means of reducing blast.

The fungus is thought to overwinter on St. Augustine grass (Stenotaphrum secundatum (Walt.) Kuntze), which is probably the chief secondary host as regards rice. Kretschmer (1954) was able to differentiate the symptoms of potassium deficiency in this grass from lesions of Piricularia oryzae. Visual symptoms attributed to potash deficiency were described as small round, to rather large, elongate, solid brown areas appearing first on the older blades. The fungus disease manifests itself by ashen-grey lesions with circular centers surrounded by brown areas. He was unable to isolate fungi from the lesions caused by potassium deficiency.

The worst epiphytotic of "leaf blast", caused by Piricularia oryzae, ever to occur in the in the United States is recorded from the Everglades Experiment Station. On July 4, 1952, diseased areas of rice plants about a yard in diameter were noted in a thickly seeded field of Zenith rice (Figure 2). These areas coalesced within 3 days, giving the field the appearance of having been severely frosted. The plants were about 6 weeks old and in the tillering stage. A few individual plants strongly resembling those of the variety Lacrosse were not affected. Varietal determination was confirmed upon maturity when seed from the mature

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[3] This world collection of rice varieties is prepared and distributed annually by Dr. C. Roy Adair, Head, Rice Section, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, with whom the author is a Collaborator. Fresh seed are made for perpetuation in the germplasm bank and agronomic characteristics are recorded.

FIGURE 2. Field of Zenith rice infected with leaf blast. Note spread of the disease from foci to larger areas which coalesce. June 1953.

FIGURE 3. Reaction of varieties to leaf blast in the USDA World Collection of Rice Varieties. Center row was killed by the disease 71 days after planting. Left -- PI 173603 Turkey; Center -- PI 182253 Turkey; Right -- CI 1600 Colusa. July 1954.

disease-free plants were planted, and the plants compared with those grown from known La-crosse seed. The agronomic characters of the plants from the two seed sources were identical, including smooth, wide leaves, smooth glumes, and identical yields.

Zenith rice planted at heavy seeding rates suffered from blast disease in commercial fields in 1953 and 1954. Plots of Zenith seeded at 22.5, 45, and 67.5 pounds per acre (equal to 1/2, 1, and 1 1/2 bushels) showed no differences in yield from plots seeded at rates of 100 and 162 pounds per acre. Incidence and severity of blast disease were lower with the lower planting rates. The difference in blast severity at the different planting rates apparently is due to local warming in the rice paddy. The lighter the rate of seeding, the later the occurrence according to the stage of rice growth of a period in which the days and nights are both warmer than the surrounding areas. This warming occurs when the leaf cover is open enough so that the water can take up abundant solar heat during the day, but dense enough to slow outward radiation at night and thus permit retention of the absorbed heat. In the Everglades this local warming usually benefits the blast fungus. The fungus is favored at an early stage of rice plant development by heavy seeding rates, whereas the lighter seeding rates do not provide favorable conditions until a later stage in plant growth (8).

DISCUSSION

The Everglades area is well known as a place where epiphytotics of many diseases on most crops can occur each year. The climate is characterized by high rainfall and high relative humidity. The muck soil is very high in organic matter and nitrogen. Dew forms almost every night in the year with relative humidity of practically 100 percent from about 9 p.m. to 9 a.m. In experimental work, artificial inoculation with spores is seldom necessary, as natural infection usually causes diseases to occur sometime during the life of plants. For these reasons, the area has been the location of many regional and national uniform and disease nurseries of grain crops. Since diseases usually manifest themselves early in the life of the crop plants, many lines or inbreds can be discarded before pollen sheds (see Figure 3).

By way of summary, certain generalizations can be made. A number of fungus diseases have been observed on rice in the Everglades. Only two have caused serious commercial losses to rice production. Seedling blight caused 10 percent fatality. It can be controlled by early flooding. Leaf and head blast caused complete crop failure at times. It was controlled by early planting to take advantage of cool night temperatures.

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THE EFFECTS OF DEEP COVERING OF ORGANIC MATTER AND NON-DIRTING WEED CONTROL ON PEANUT STEM ROT

Kenneth H. Garren and George B. Duke

Abstract

Over a 3-year period, under Virginia conditions, the deep covering of organic matter on land prior to planting peanuts followed by a type of cultivation that avoided bringing soil in contact with the plants effectively controlled the stem rot disease caused by Sclerotium rolfsii Sacc. The avoidance of bringing soil in contact with the above ground parts of the plants had a greater effect on stem rot control than did the deep covering of the organic matter prior to planting. However, in a field heavily infested with S. rolfsii, the deep covering reduced the proportion of stem rot affected plants by about 50 percent.

The use of these two cultural practices resulted in increased yields. There was a consistent inverse relation between stem rot and yield, and it was assumed that these yield increases resulted from control of stem rot. The effect of the treatments on quality of the harvested crop was neither striking nor consistent. Quality was usually higher in plots receiving deep covering. There sometimes were minor decreases in quality which could be associated with the non-dirting treatment but these were not of sufficient magnitude to offset the beneficial effects of non-dirting treatment on yield.

INTRODUCTION

The stem rot disease of peanuts (Arachis hypogaea L.) caused by Sclerotium rolfsii Sacc. is an old and persistent problem found in most peanut producing areas. In a recent paper Garren and Duke (5) reviewed the problem and reported on 2 years' field work in Virginia. They found that a combination of two cultural practices gave considerable promise as an effective control program for this disease. These practices were: (a) plowing in such a manner that the organic matter on the surface of the soil was buried to a depth of 4 to 8 inches; and (b) cultivation without throwing soil around the base of the peanut plants. In the preparation of their paper Garren and Duke considered many suggestions for concise descriptive names for these practices and their opposites. All of these names were found to have some obvious shortcomings. The terms "deep covering" and "non-dirting" were finally chosen as least likely to cause prolonged controversy. The terms now assigned the opposite practices are: "surface mulching[1]" and "dirting."

Garren and Duke (5) discussed the history of these two practices in some detail, and a brief outline of this discussion follows: Taubenhaus in 1919 (6) recommended deep plowing as a control measure for diseases caused by S. rolfsii. In 1918 Tisdale (7) noted that S. rolfsii is such a vigorous saprophyte that organic matter in the soil and organic debris on the surface of the soil should be considered when management practices are developed for areas with a high degree of S. rolfsii infestation[2]. Apparently Boyle (1) in 1952 first publicized the merits of non-dirting cultivation. In an abstract of a talk given that year he stated: "Pre-emergence compounds and flat culture have given encouraging results." This abstract also first recommended using a combination of two practices corresponding closely to deep covering and non-dirting in attempting to control peanut stem rot.

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[1] Three years' observation showed that in the treatment called "shallow covering" in the 1957 publication (5) most of the organic matter remained on the surface and the remainder was incorporated into the upper 2 inches of the soil. "Surface mulching" is now considered to be a better name for this treatment.

[2] The two specific measures recommended by Tisdale for rice fields were early plowing followed in a few weeks by thorough disking. They are not mentioned in reports of studies of control of S. rolfsii after 1921. Perhaps they have not been investigated further or perhaps they are effective only in the peculiar culture of rice.

A report from Georgia by Boyle and Hammons (2) was reviewed only briefly by Garren and Duke since it appeared after the Virginia report was submitted for initial editing. Boyle and Hammons stated: "The results of a single experimental test of simple design are used to illustrate some fundamental points in the development and selection between methods of culture for peanuts." Procedures described in the Georgia report are essentially the same as those used in Virginia, and the headings "moldboard" and "disk", "cultivated" and "herbicide", used in the tables of the Georgia report correspond, in order, to the treatments "deep covering" and "surface mulching", "dirting" and "non-dirting", of the Virginia study. Although "herbicide" was the name of a treatment in the Georgia report, the authors stated: "The use of a pre-emergence herbicide appears a most practical method to avoid smothering the lower part of the plant. For some who may not choose to use the herbicide, certain cultivating tools used with truck crops may have possibilities in the solution of this problem." Boyle and Hammons used the disk-cultivated treatment as the base, and reported yield increases of 32 percent for moldboard-herbicide treatment, 25 percent for moldboard-cultivated treatment, and 12 percent for disk-herbicide treatment. They stated that conventional methods of planting and cultivating not only favored the development of stem rot but also checked normal development of Spanish peanut plants by suppressing lateral branching and reducing flowering.

Results obtained in Virginia in 1957 furnish additional proof of the effectiveness of both the deep covering of organic matter and the non-dirting type of cultivation as control measures for peanut stem rot. This paper summarizes 3 years' work on these cultural practices by adding the 1957 data to that previously reported in 1955 and 1956 (5). It will not touch upon the other lines of investigation described in the first report (5).

PROCEDURE

This experiment was designed to provide the greatest possible contrasts for both deep covering of organic matter and the non-dirting type of cultivation in order to determine whether either or both of these cultural practices is particularly effective in controlling stem rot (5). The "word-of-mouth" history of the field used in 1956 is that it has long been regarded as a poor field for peanuts because of two diseases -- stem rot and a pod rot of as yet undetermined cause. The results for 1956 (5) showed that the field was severely infested with S. rolfsii. This presented an excellent opportunity to test the effectiveness of these cultural practices under a most extreme condition -- repetition of peanuts without rotation on a field obviously badly infested with S. rolfsii. Consequently the 1956 split plot test "A" of 32 subplots in 8 replications described by Garren and Duke (5) was repeated on the same field in 1957 with each subplot located on exactly the same spot in 1957 as in 1956. Again the Virginia Bunch 46-2 variety was used. Procedures for 1957 were as described for 1956 (5). The treatments for the main plots were (a) deep covering of surface litter and sclerotia and (b) surface mulching of surface litter and sclerotia, and for the subplots (x) non-dirting cultivation and (y) dirting cultivation. In the non-dirting treatment weed control in the drill row was accomplished by the application of DNBP (4,6-dinitro ortho secondary butyl-phenol) 3 days after planting, supplemented by hand pulling of weeds when necessary. In the dirting treatments weeds were controlled by the conventional procedure of throwing dirt around the bases of the plants.

The same kinds of data were taken in 1957 as in 1956 (6).

RESULTS AND DISCUSSION

The results are presented in Table 1, 2, and 3 and Figures 1, 2, and 3. Figure 1 shows the data of Table 1 in graphic form and Figure 3 presents the data of Table 2 in graphic form. In considering these data it should be kept in mind that this experiment was designed to provide the aforementioned broad contrasts. For this reason the most important data are those on infection. There is no sound basis for comparing the yields of this experiment with yields of other types of experiments or with yields obtained by farmers. Because of the many factors which influence yield, the yield data in this experiment should be considered within years rather than compared between years. Thus the percentage increases in yield of Figure 2 are more pertinent to the main purpose of the experiment than are the yields themselves.

Since the experiment was duplicated on the same site in 1956 and 1957 there is a natural tendency to compare 1957 yields with 1956 yields, particularly since the 1956 yields were remarkably high. It was realized at the outset that decreased yields and increased pod rotting and other damage might reasonably be expected from repeating the experiment without ro-

Table 1. Prevalence of Sclerotium rolfsii infection on Virginia Bunch 46-2 peanuts, and yields 1955 through 1957, Holland, Virginia.

PERCENT OF STAND INFECTED

	1955			1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	5.1	7.8	6.5	20.0	39.2	29.6	46.4	75.8	61.1
Non-Dirting	0.8	0.8	0.8**	4.8	10.1	7.4**	4.7	16.2	10.4**
Mean	3.0	4.3		12.4**	24.6		25.5**	48.0	
	Interaction			Interaction*			Interaction*		

YIELD IN POUNDS PER ACRE

	1955			1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	2694	2729	2711	3247	2322	2784**	1632	1094	1365
Non-Dirting	2924	3134	3029**	4142	3698	3920**	2173	1717	1945**
Mean	2790	2931		3695	3010		1904*	1405	
	Interaction			Interaction*			Interaction		

** Significant at 1% level. * Significant at 5% level.

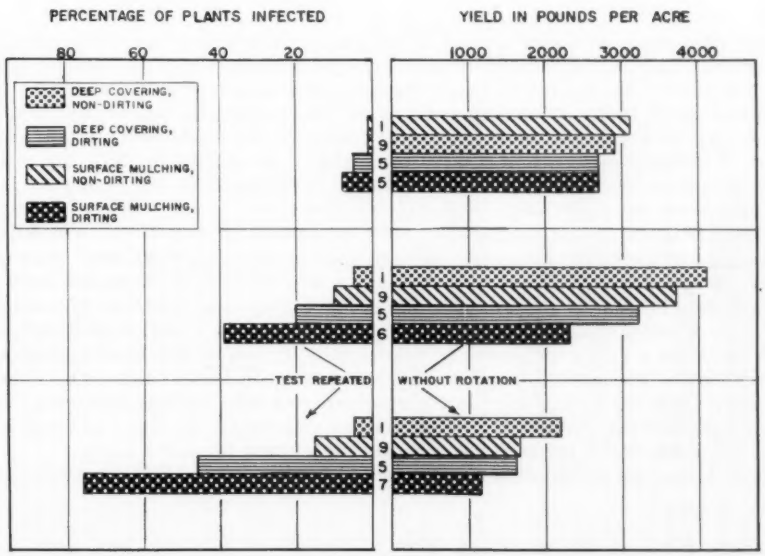


FIGURE 1. Relation of infection by Sclerotium rolfsii to yields of Virginia Bunch 46-2 peanuts for 4 treatments and 3 years in Virginia.

Table 2. Virginia Bunch 46-2 peanuts left in the soil at digging, 1956 and 1957, Holland, Virginia.

POUNDS OF NUTS PER ACRE

	1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	773	1210	991	837	1298	1067
Non-Dirting	238	826	532**	528	862	695**
Mean	505*	1018		682*	1080	
	Interaction			Interaction		

PERCENTAGE OF THE NUTS WHICH WERE DECAYED[a]

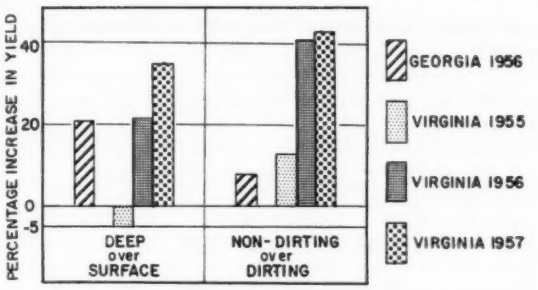
	1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	37.1	60.7	48.9	75.5	75.6	75.5**
Non-Dirting	31.0	65.6	48.3	83.7	85.9	85.0
Mean	35.6**	62,7		78.7	79.7	
	Interaction			Interaction		

** Significant at the 1% level.
* Significant at the 5% level.
[a] The nuts were counted, but only sound nuts were weighed and weight of decayed nuts was determined by a factor derived from the weight of the sound nuts.

tation on the 1956 site. In Table 1 and Figure 1 a considerable reduction in yield from 1956 to 1957 is evident. Comparing the 1957 yields plus the peanuts left in the soil in 1957 with the same data for 1956 (Tables 1 and 2) shows that the total weight of nuts formed in 1957 was not nearly as great as in 1956. It is believed that this decrease was due in part to the lack of rotation and in part to a drought lasting from the fourth to the fourteenth week of the 1957 growing season. A definite increase in pod rot was evident from 1956 to 1957. It is possible that the pod rot organism or complex was also a factor in decrease in nut formation in 1957.

It appears from the percentage of plants infected (Table 1 and Figure 1) and percentage increase in yield (Figure 2) that the lack of rotation between 1956 and 1957 actually accentuated the effectiveness of deep covering and non-dirting in controlling stem rot. It is noteworthy that the percentage of infection remained the same in 1957 as in 1956 in the deep covering non-dirting treatment, increased somewhat in the surface mulching non-dirting treatment, but doubled in both of the dirting treatments. Table 1 and Figure 1 show that for 3 years in Virginia there has been a lower incidence of infection by S. rolfsii and higher yields when non-dirting weed control was used than when dirting weed control was used. The same is true for 2 years of deep covering of organic matter as compared with surface mulching. The differences in favor of deep covering of organic matter are not so striking as those in favor of non-dirting cultivation, but differences for both were highly significant for the 2 years.

In Figure 1, yield and infection are presented in one graph. The lengths of the yield bars



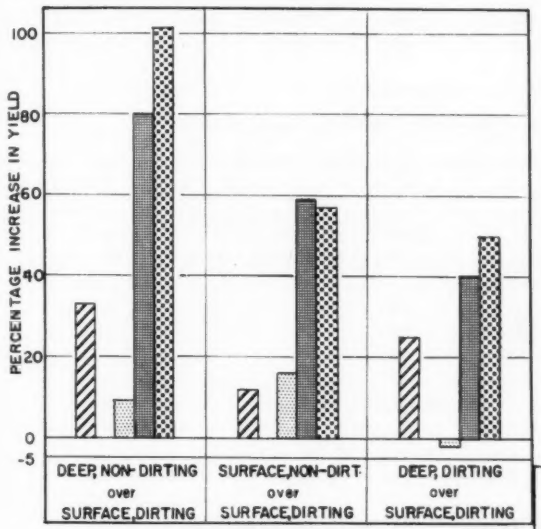


FIGURE 2. Yield of Dixie Spanish in Georgia, 1956, and yields of Virginia Bunch 46-2 peanuts for 3 years in Virginia, expressed as percentage increase of indicated treatment over base of a contrasting treatment. (Georgia data from Boyle and Hammons (2)).

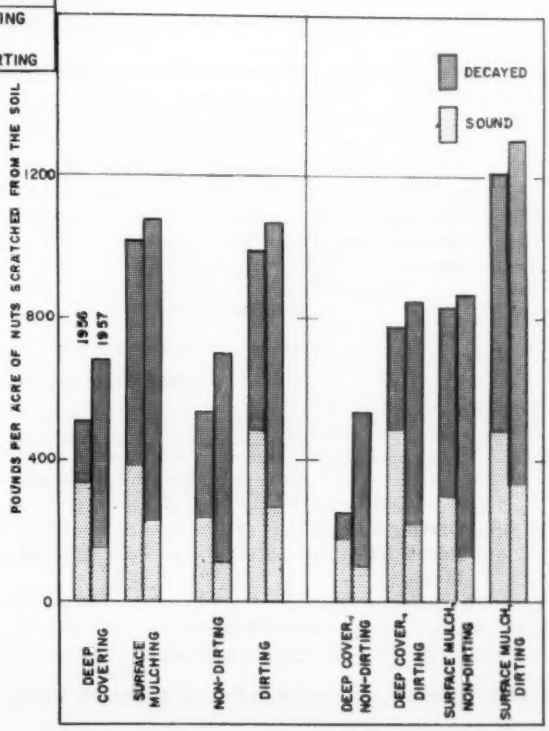


FIGURE 3. Pounds per acre of nuts left in the soil at digging following the use of the different treatments in Virginia, 1956 and 1957.

Table 3. Effect of cultural practices on quality of Virginia Bunch 46-2 peanuts, 1956 and 1957, Holland, Virginia.

Part I

PERCENTAGE OF FANCY PODS

	1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	82	84	83	86	86	86**
Non-Dirting	83	82	82	80	77	78
Mean	82	83		84	82	
	Interaction			Interaction		

PERCENTAGE OF EXTRA LARGE KERNELS

	1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	47	42	45	46	41	35
Non-Dirting	53	47	50**	34	36	43**
Mean	50*	45		40	37	
	Interaction			Interaction**		

Part II

PERCENTAGE OF SOUND MATURE KERNELS (SMK's)

	1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	64	66	66	68	66	67
Non-Dirting	71	64	67	67	65	66
Mean	70*	65		67*	65	
	Interaction			Interaction		

PERCENTAGE OF DAMAGED KERNELS

	1956			1957		
	Deep Covering	Surface Mulching	Mean	Deep Covering	Surface Mulching	Mean
Dirting	2.2	2.7	1.8	1.6	3.1	2.3
Non-Dirting	1.3	2.2	1.8	3.0	4.1	3.5
Mean	1.7*	2.3		2.3*	3.6	
	Interaction			Interaction		

** Significant at the 1% level. * Significant at the 5% level.

and the infection bars are almost always inversely proportional so that an inverse relation between yield and infection is apparent without determining a correlation coefficient. This relation is much clearer when the degree of infection is high as in 1956 and 1957, than when the degree of infection is low as in 1955. Therefore, the writers feel that the practices of dirting and surface mulching were detrimental to yield largely because they furnished a highly favorable environment for the stem rot organism.

The greater effectiveness of non-dirting is more evident when treatment combinations are considered. Throughout the 3 years the least infection was found in subplots receiving both deep covering and non-dirting, with surface mulching and non-dirting next most effective. Nevertheless, the reduction in infection which was obtained from deep covering was highly significant when the field was badly infested with S. rolfsii.

Examination of plants approaching maturity indicated that an extremely high percentage of the peg rots was caused by S. rolfsii. The data of Table 2, therefore, are further substantiation of these comparative effects of treatments and treatment combinations on infection by S. rolfsii.

The effectiveness of these practices in controlling stem rot is particularly emphasized by expressing yields as percentage increases over some base (Figure 2). Comparable data from the Georgia report (2) also used in Figure 2 suggest that the beneficial effects of deep covering on yield may be greater in Georgia than in Virginia. The Virginia results for 1956 and 1957 indicate that where there is a moderate or high S. rolfsii infestation potential, the practices of non-dirting and deep covering may both result in considerably increased yields.

The use of a herbicide may be almost essential to non-dirting cultivation on a large scale. DNBP, used as a herbicide in these tests, is also used as an insecticide; thus it is a general toxin. Chappell and Miller (3) working in this same section of Virginia found indications that the chemical can be toxic to S. rolfsii in the field. A separate study of the possible direct effects of DNBP on S. rolfsii is underway (5), and this aspect is not considered in this report.

The data on quality (Table 3) were obtained by established sampling procedure. Some doubt has been cast upon the validity of these procedures (4) but the uniformity of effects within years shown in Table 3 suggests that they are not in error. In 1956 and 1957 there were indications that quality was higher in peanuts from plots receiving deep covering. In 1957 there were a few aspects in which quality was higher in peanuts from plots which received dirting.

A great deal of pod rot was evident at digging in 1957 in many of the plots. The data of Table 2 also show a considerable increase in rotted pods from 1956 to 1957. The fairly high proportion of damage (Table 3) in the treatments with 5 percent and 16 percent, as opposed to slightly lower proportion of damage in the treatments with 46 percent and 76 percent, of the plants infected with S. rolfsii indicates that the fungus was not involved in this damage. Table 2 shows that the proportion of rotted pods among pods left in the soil in 1957 was higher in the treatments with the lower infection by S. rolfsii than in treatments with the higher infection. This indicates that the organism (or organisms) causing pod rot was favored more by conditions in the treatments that were more effective, than by conditions in the treatments that were less effective, in controlling stem rot. Pod rot definitely confused the quality picture in 1957.

CONCLUSIONS

Results of 3 years' experimental work in Virginia showed that a combination of deep covering of organic matter with non-dirting cultivation (weed control in which soil is not thrown to the base of the peanut plants) can be an effective control program for the stem rot of peanuts caused by S. rolfsii.

In Virginia, apparently, the non-dirting cultivation practice was responsible for the greater portion of the effectiveness of this combination. When the test was on a field badly infested with S. rolfsii, however, deep covering of organic matter also was highly effective in suppressing the disease.

An inverse relation between infection and yield is evident from consideration of effects of these treatments either individually or in combination. For these 3 years in Virginia the main beneficial effects of these cultural practices was their retarding effects on stem rot development and the increased yield of pods associated therewith.

These cultural practices had no striking or consistent effect on the quality of the harvested crop.

ACKNOWLEDGMENTS

Many co-workers have contributed to this study. The writers wish to acknowledge especially the assistance of: Victor R. Boswell and Wallace K. Bailey, Crops Research Division, Agricultural Research Service, Beltsville, Maryland, who made many valuable suggestions; Clyde Y. Kramer, Virginia Agricultural Experiment Station, Blacksburg, Virginia, who made the statistical analyses and advised on experimental design; and S. A. Wingard, Virginia Agricultural Experiment Station, Blacksburg, Virginia, who contributed general and editorial advice.

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DISEASES OF FORAGE CROPS UNDER SPRINKLER IRRIGATION IN THE SOUTHEAST

E. A. Curl and H. A. Weaver[1]

Abstract

During July and August, 1954 and in each month of 1955, the severity of diseases that occurred on sprinkler-irrigated forage crops was estimated. These crops were: Kenland red clover, intermediate white clover, Oregon Ladino clover, Atlantic alfalfa, African alfalfa, commercial orchardgrass, Kentucky 31 fescue, and Reed canarygrass. At least 18 fungal pathogens were isolated and two virus diseases recognized on the eight crops. More diseases were present in May, June, and July, part of the 5-month period during which most water was applied, than in other months. Pseudoplea leaf spot (Pseudoplea trifolii (Rostr.) Petr.) was the most persistent and damaging disease of the white clovers. Stemphylium leaf spot (Stemphylium botryosum Wallr.) and rust (Puccinia rubigovera tritici (Eriks.) Carleton) were the most damaging diseases on alfalfa and grasses, respectively. For most of the crops, disease severity was slightly greater in plots that received the most water. The diseases which showed the greatest increase in severity under high-level irrigation during a 1-year period were Pseudoplea leaf spot on intermediate white clover, yellow patch (virus) and Curvularia leaf spot (Curvularia trifolii (Kauf.) Boed.) on Ladino clover, and Pseudopeziza leaf spot (Pseudopeziza medicaginis (Lib.) Sacc.) on African alfalfa. The severity of powdery mildew (Erysiphe polygoni DC.) on red clover and of rust on orchardgrass was greater in in the low-level irrigated plots.

In the past little attention has been given to the effects of irrigation on development of plant diseases. The increasing use of irrigation for growing forage legumes and grasses in Alabama and other parts of the Southeast might be accompanied by an increase in disease problems.

The following disease study was done in cooperation with the Departments of Agronomy and Soils and Agricultural Engineering of the Agricultural Experiment Station and with the Agricultural Research Service, United States Department of Agriculture. It is a part of a long-range screening program to determine the adaptability of certain forage crops for irrigation under climatic conditions characteristic of the Southeast.

METHODS

The experimental plots used in the study were located on the Thorsby Foundation Seed Stocks Farm in Chilton County, Alabama. In October, 1954, the area was prepared, fertilized, fumigated with Dowfume W-85 nematocide, and seeded with the eight forage crops listed in Table 1. Each crop was replicated four times, and each replication was divided into three subplots by metal plot borders. Each subplot measured 6 by 6 feet.

Individual subplots were irrigated differentially from April through August by means of a plastic hose system leading from portable pipes and terminating at sprinkler nozzles on movable tetrapod supports. The water source was a stream nearby. The frequency of irri-

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Table 1. Total amount of water in inches received by low- and high-level irrigated plots of forage crops during the period of differential irrigation.

Month 1956	Red clover		Ladino clover		Intermediate white clover		Atlantic alfalfa		African alfalfa		Orchard-grass		Kentucky fescue		Reed canary-grass	
	Low[a]	High[b]	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
April	.50	7.15	.50	7.15	.50	7.15	.50	7.15	.50	7.15	.50	6.75	.50	6.75	.50	6.75
May	2.50	7.23	2.50	7.23	2.50	7.23	5.25	7.23	5.25	7.23	4.25	7.23	4.25	7.23	4.25	7.23
June	2.75	3.32	3.33	4.32	3.33	4.32	0.00	3.82	0.00	5.87	2.30	4.32	2.30	4.32	2.30	3.32
July	4.25	5.08	4.25	6.33	4.25	6.33	4.25	6.33	4.25	6.33	3.75	5.08	3.75	5.08	3.75	5.08
August	0.00	6.00	1.75	7.50	1.75	7.50	2.75	6.90	2.75	6.90	0.00	6.00	0.00	6.00	0.00	6.00
Total	10.00	28.78	12.33	32.53	12.33	32.53	12.75	31.43	12.75	33.08	10.80	29.38	10.80	29.38	10.80	28.38

[a]Irrigation water only.
[b]Irrigation water + natural rainfall.

gation and the amount of water applied were determined by soil sampling. The dryest subplots, which received only enough water to maintain the plants, were protected from natural rainfall during the period of differential irrigation by plastic-covered frames which were placed over the plots when rains occurred. The total amounts of water received by high- and low-level irrigated plots are given in Table 1. All plots were clipped several times during the period March to September, 1956 for yield determinations. However, no attempt was made to relate clipping frequency or forage yields with disease severity.

Only subplots 1 and 3, the dryest and wettest treatments, respectively, were used for disease studies. The plots were examined at least twice each month. Diseases that appeared to be different, on the basis of kind of symptom, were given temporary letter designations, and the severity of each disease was estimated visually. Representative diseased specimens were taken to the laboratory for isolation and positive diagnosis.

A numerical rating system[2] for disease severity was arranged as follows:

1 = Clean; apparently no disease present.
2 = Up to 10 percent of leaves affected but no appreciable quantity of tissue destroyed.
3 = 10-50 percent of leaves affected and/or up to 25 percent of tissue destroyed.
4 = 50-90 percent of leaves affected and/or 25-50 percent of tissue destroyed.
5 = Over 90 percent of leaves affected and/or over 50 percent of tissue destroyed.

Obviously, some diseases that affect entire leaf surfaces do not always destroy much leaf tissue. Examples are virus diseases and powdery mildew. Other diseases, such as stolon, stem, and crown rots, could not be rated by the system above. The severity of stolon rot caused by Sclerotium rolfsii Sacc. was estimated by measuring the diameters of areas of killed stolons in the plots, and the severity of crown rot caused by Colletotrichum trifolii Bain & Essary was estimated by counting the total number of dead plants in each plot.

In 1955, numerical data were recorded only for July and August. During these two months, disease severity was estimated for the high and low irrigation levels of all four replications of the eight forage crops. It was found that the numerical ratings for a given irrigation level seldom varied between replications of any single crop. Therefore, in 1956, numerical estimates were limited to include a single replication of two irrigation levels in the eight crops, and an intensive study was conducted of diseases that occurred over a period of 1 year. At the same time, all other replications were thoroughly examined for unusual disease occurrences or variations from the intensively studied plots.

DISEASES IN 1955

In Table 2 is a list of pathogenic fungi which were isolated during July and August, 1955, from eight forage crops subjected to two levels of irrigation. The severity of the diseases caused by these fungi on both low- and high-level irrigation plots of the eight forage crops are given in the last two columns as the averages of all the numerical readings made on the four replications during the 2 months. Also shown in Table 2 are estimates of the relative severity of diseases averaged over both irrigation levels for each of the 2 months, July and August. Since a numerical rating scheme as used here is subjective, and may vary in level from one reading period to the next due to vagaries of the reader, the averages from one time period to another are given only as + = light, ++ = medium, +++ = severe rather than the actual average of numerical readings.

Only very small differences in disease severity were detected between the two irrigation levels in July and August, 1955. Most prevalent of the diseases were Stemphylium leaf spot (Stemphylium botryosum Wallr.) and common or Pseudopeziza leaf spot (Pseudopeziza medicaginis (Lib.) Sacc.) on the two alfalfas. Also, a large number of plants in the African alfalfa plots were killed by Colletotrichum trifolii.

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[2] A disease rating system was suggested by E. F. Schultz, Jr., Biometrician of the Agricultural Experiment Station.

Table 2. Pathogenic fungi occurring on forage crops under two levels of irrigation during July and August, 1955, and the relative severity of diseases caused by them.

Crop	Cause of disease	Relative severity of disease[a]		Average disease Ratings for 2 months	
		July	Aug.	Low-level irrigation	High-level irrigation
Intermediate white clover	Pseudoplea trifolii	+	+	2.0	2.3
	Colletotrichum graminicola	+	+	2.0	2.0
	Curvularia trifolii	+		1.3	1.3
	Cymadothea trifolii		++	2.2	2.2
	Sclerotium rolfsii	+	+	---	---
Oregon Ladino clover	Colletotrichum graminicola	+	+	1.9	1.9
	Pseudoplea trifolii	+		1.3	1.5
	Curvularia trifolii		+	1.5	1.4
	Colletotrichum destructivum		+	1.5	1.5
	Sclerotium rolfsii	+	+	---	---
Kenland red clover	Colletotrichum graminicola	+	+	1.4	2.0
	Erysiphe polygoni	+		1.0	1.5
Atlantic alfalfa	Stemphylium botryosum	++	++	2.8	3.0
	Pseudopeziza medicaginis	++	++	2.8	3.0
	Colletotrichum trifolii	+	+	---	---
African alfalfa	Stemphylium botryosum	++	+++	3.3	3.7
	Pseudopeziza medicaginis	++	+++	3.3	3.6
	Colletotrichum trifolii	++	++	---	---
Commercial orchardgrass	Helminthosporium sp.	+	+	2.0	2.0
Kentucky 31 fescue	Helminthosporium sp.	+	+	1.7	1.8
Reed canary-grass	Helminthosporium sp	+	+	1.5	1.5

[a] Estimates were obtained from combined averages of figures for low- and high-level irrigation in 4 replications; + = light; ++ = medium; +++ = severe.

Table 3. Pathogenic agents occurring on forage crops under two levels of irrigation during 1956 and the relative severity of diseases caused by them.

Cause of disease	Relative severity of disease[a]												Average disease ratings for year at 2 irrigation levels	
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Low level	High level
Kenland red clover														
Cercospora zebrina							++	+	+				1.6	2.0
Erysiphe polygoni				+	+	+	+	+			+		2.0	1.3
Pseudoplea trifolii	+	+	+							+	+	+	2.0	2.1
Colletotrichum destructivum	+	++	+	++	++	+							2.2	2.5
Virus						+					+	+	1.7	1.8
Oregon Ladino clover														
Curvularia trifolii						+	+	++	+	+	+	+	1.7	2.3
Cercospora zebrina							+						2.0	1.0
Pseudoplea trifolii	+++	+++	++	+	+	+				+	+	++	2.4	2.6
Sclerotium rolfsii						+++	++						—	—
Colletotrichum destructivum	+	+	+	+	++	+	+						1.9	2.0
C. graminicola				+	+	+	+						1.4	1.6
Stagonospora sp.				+	+	+							1.8	1.7
Stemphylium sp.						+	+					+	1.5	1.7
Virus (yellow patch)			+	+	+	+	+	+		+			1.2	1.9
Intermediate white clover														
Cercospora zebrina					+	++	++	++	+	+	+	+	2.1	2.4
Pseudoplea trifolii		++	++	++	++	++				+	+	++	2.4	3.0
Curvularia trifolii						+	++			+	+	+	1.4	1.7
Sclerotinia trifoliorum		++											—	—
Sclerotium rolfsii						+	+	+					—	—
Colletotrichum destructivum	++	+	+	+	+	+							1.8	1.8
C. graminicola					+	+							1.8	1.8
Stagonospora sp.													1.0	1.5
Ascochyta sp.		+											1.5	1.5
Virus (yellow patch)			+	+		+	+	+	+	+			1.4	1.6
Uromyces trifolii												+	1.5	1.5
Stemphylium sp.												+	2.0	2.0

Table 3 (continued).

Cause of disease	Relative severity of disease[a]												Average disease ratings for year at 2 irrigation levels	
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Low level	High level
Atlantic alfalfa														
Stemphylium botryosum					+	++	++	++	+	+	++	++	2.6	2.7
Pseudoplea trifolii	++	++	+									+	2.3	2.3
Pseudopeziza medicaginis		+	++	++	+	+							1.8	2.4
Uromyces striatus medicaginis						+	+		+	+	++	+	2.0	2.1
Colletotrichum destructivum					+								1.5	1.5
Sclerotinia trifoliorum			+										—	—
Ascochyta sp.		+			+								1.5	1.5
Virus						+	+	+					1.3	1.3
African alfalfa														
Stemphylium botryosum					++	++	+	+	+	+	++	++	2.4	2.7
Pseudoplea trifolii	+++	+++	+			+					+	+	2.3	2.3
Pseudopeziza medicaginis			+++	++	+	+		+					2.1	2.8
Colletotrichum graminicola					+								1.5	1.5
Sclerotinia trifoliorum			+										—	—
Ascochyta sp.		+	+			+	+						1.6	1.7
Virus								+				+	1.5	1.5
Commercial orchardgrass														
Helminthosporium sp.			+	+	++	+	+	+					2.3	2.1
Puccinia rubigo-vera tritici						+	+++	+++	+	+	+++	+++	3.5	3.3
Curvularia sp.						+	+	+					1.7	1.7
Kentucky 31 fescue														
Colletotrichum graminicola	+	+	+		+								2.0	2.1
C. destructivum				+		+							1.5	1.5
Helminthosporium sp.				+	++	+	++			+			2.2	1.9
Puccinia rubigo-vera tritici					+	+	+	+	+				1.8	1.9
Reed canarygrass														
Colletotrichum destructivum	+												2.0	2.0
Helminthosporium sp.				+	+	+	+						1.9	2.0
Puccinia rubigo-vera tritici						+							1.0	2.0
Curvularia sp.								+					2.0	2.0
Ascochyta sp.		+		+									2.0	3.0

[a] + = light; ++ = medium; +++ = severe.

DISEASES IN 1956

During 1956, at least 18 fungal pathogens were isolated or identified from plant material of the eight forage crops under irrigation. In addition to these, two virus diseases, one on white clover and one on alfalfa, were recognized. Table 3 shows the pathogenic agents that were found to be associated with each crop and the relative severity of the diseases caused by them during each month of a 1-year period. The Table also shows the comparative average numerical severity ratings of each disease under two levels of irrigation for the entire year.

Considering all of the eight crops together, more diseases were present in May, June, and July than in the other months. These were 3 of the 5 months in which the plots received the most water (Table 1). The severity of disease varied with the kind of disease and the season favoring its rapid development. It is apparent in Table 3 that Pseudoplea leaf spot (Pseudoplea trifolii (Rostr.) Petr.) persisted throughout most of the year on the white clovers, but it was most damaging during January, February, and March. Stemphylium leaf spot, and rust (Puccinia rubigo-vera tritici (Eriks.) Carleton) were the most damaging diseases on the alfalfas and on orchardgrass, respectively. Rust was slightly more severe in the drier plots.

The differences in disease severity between crops under low-level and high-level irrigation were small but consistent for most diseases. Among the annual averages, the figures for five diseases suggested important differences between low- and high-level irrigation treatments. These were powdery mildew (Erysiphe polygoni DC.) on red clover, yellow patch (virus) and Curvularia leaf spot (Curvularia trifolii (Kauf.) Boed.) on Ladino clover, (Pseudoplea leaf spot on intermediate white clover, and Pseudopeziza leaf spot on African alfalfa. Of these, only powdery mildew on red clover was more severe in the drier plots. The figures for Cercospora zebrina Pass. on Ladino clover and for Ascochyta sp. on Reed canarygrass suggested considerable differences between levels of irrigation. However, Cercospora did not occur frequently enough to obtain a reliable average, and Ascochyta was obtained only in laboratory culture from leaves which showed no external evidence of the disease. Rust (Uromyces striatus medicaginis) on Atlantic alfalfa occurred during most of the period June to December, but caused little damage. Several other fungi were isolated from leaf spots which appeared to be primarily the result of infection by still other fungi. Since they caused no noticeable damage they were given the low rating of "2". These were Colletotrichum destructivum O'Gara on red clover; C. destructivum and C. graminicola (Ces.) Wils. on Ladino clover; C. destructivum, C. graminicola, Ascochyta sp., and Stemphylium sp. on intermediate white clover; C. destructivum and Ascochyta sp. on Atlantic and African alfalfas; and Curvularia sp. and Ascochyta sp. on Reed canarygrass. Other organisms frequently isolated from leaf spots apparently caused by pathogenic fungi listed in Table 3 were species of Alternaria, Fusarium, Botrytis, Rhizopus, Xylaria, Penicillium, Dinemasporium, Trichoderma, Aphelenchus (nematode), and a number of non-sporulating and unidentified fungi and bacteria. Alternaria sp. was isolated consistently from all plants brought to the laboratory.

DISCUSSION

On the basis of the data, it appears that most of the leaf spotting diseases occurring on the eight forage crops tended to increase in severity under high-level sprinkler irrigation. The annual average disease ratings for 1956 showed small but consistent increases. Differences between low- and high-level irrigation were usually more pronounced for individual examination dates than for the annual averages. One- and two-figure differences between irrigation levels for specific diseases were common. The most striking diseases in 1956 were rust on orchardgrass, Pseudoplea leaf spot on the white clovers and Stemphylium leaf spot on the alfalfas. Several of the fungi isolated from white clover were the same as those isolated previously by Garren[3].

In isolating from leaf spots of the various forage crops, it was noted that, despite surface disinfestation with 1:1000 mercuric chloride, one to four other fungi or bacteria invariably were isolated along with those that appeared to be the primary pathogens. Of these other fungi, species of Fusarium and of Alternaria appeared most frequently in the plates. The role of these secondary invaders in contributing to the total damage inflicted upon forage plants is

[3] Garren, Kenneth H. 1954. Disease development and seasonal succession of pathogens of white clover. Part I. Leaf diseases. Plant Dis. Reptr. 579-582.

difficult to evaluate.

Two soil-borne diseases of importance occurred in 1956. Sclerotium rolfsii was common in white clover plots during June, July, and August. More abundant growth of the fungus occurred under high-level irrigation, but the sizes of areas of killed plants seemed to differ little from those under low-level irrigation. Sclerotinia trifoliorum Eriks. was seen in intermediate white clover in February and in the alfalfa plots in March. Damage was negligible, and the disease occurred too infrequently to compare effects of irrigation levels.

Other observational notes of interest were recorded during the year. Stemphylium leaf spot damage on alfalfa was more severe in January and February, following cold injury which occurred in January. African alfalfa seemed particularly susceptible to cold injury. Cold periods in the spring also were followed closely by the appearance of S. trifoliorum in alfalfa and white clover plots. The yellow patch virus disease of white clover was present during most of the time from March to October. It did not cause damage that could be detected visually, but it seemed to undergo renewed activity after each clipping. Powdery mildew first appeared in the red clover plots late in April and was prevalent through August. This disease seldom occurred in the high-level irrigated plots.

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STUDIES ON THE CONTROL OF BROOMRAPE ON TOMATOES, SOIL FUMIGATION BY METHYL BROMIDE IS A PROMISING CONTROL

Stephen Wilhelm[1], Lee C. Benson[2], and James E. Sagen[3]

Abstract

Attempts to kill the minute seed of Orobanche ramosa L. in soil have been successful only by fumigation with methyl bromide. Methyl bromide, when applied by a mechanical circulator under polyethylene tarpaulins to moist, listed soil at rates above 1/2 pound per 100 square feet, has generally reduced the infestation of broomrape in 1/4-acre plots to fewer plants than occurs in 1 square foot of nonfumigated soil. Commercial Weedfume, a solution of methyl bromide, gave excellent broomrape control when chiseled into the soil by tractor equipment at rates above 175 pounds of actual methyl bromide per acre followed immediately by tarping.

The branched broomrape, Orobanche ramosa L., a flowering plant, parasitic on the roots of many plants, is a serious pest of tomato in Alameda County, California. Plants heavily parasitized early in development rarely produce an economic crop of fruit. Resistance to broomrape has not been noted in tomato nor reported in the literature available thus far to the authors. In this study the tomato varieties San Marzano, Red Top, Early Pack, Ace, and Pearson were all susceptible. San Marzano, Red Top, Early Pack, and Ace appeared to be more severely affected than Pearson or John Moran. The latter variety is grown extensively in the area of California infested with broomrape.

Studies of the past 4 years have confirmed the fact, discovered by Lee C. Benson in an earlier test, that methyl bromide applied to soil under polyethylene tarpaulins at rates of 0.5 pounds per 100 square feet and higher is effective in killing broomrape seeds. These studies have also demonstrated the ineffectiveness of many other materials. Benson's earlier tests showed that the following materials were ineffective in controlling broomrape: calcium cyanamide applied to land to include rates of 1000 pounds per acre, Crag Herbicide-1 (90% sodium 2,4-di-chlorophenoxyethyl sulfate) applied at rates of 2, 4, and 6 pounds per acre, Shell CBP-55 (1 chloro-3 bromopropene-1) applied at 30 gallons per acre in single and split-injection applications and as a 38% emulsion to the soil surface, and Shell D-D (a mixture of mainly 1:3 dichloropropene and 1:2 dichloropropane) injected at rates to include 40 gallons per acre.

Orobanche ramosa has been recognized as growing in association with cultivated plants since early times. Bauhin (3) in 1651 described the "smaller or branched broomrape with purple flowers" as common in hemp fields in Northern Italy and in Switzerland, and what we assume to have been the same species was described in the Herbal of L'Ecluse (10) in 1601 as Orobanche polyclonos.

Koch (9) 1887, Garman (5) 1890, Hitier quoting Lavergne (8) 1894, Halsted (7) 1902, Roger (13) 1902, Selby (14) 1904, and Noffray (12) 1909, were among the first to report Orobanche ramosa on tomato. O. ramosa was first reported in California in 1929 (1). It may have been brought to California with hemp seed (17) or as a contaminant of nursery soil (15). The present population of O. ramosa in California appears to have been derived from a single small introduction because it does not manifest the full range of variation in flower color that is typical of the species elsewhere (16). Flowers here range in color from nearly white to pale blue and violet.

A two-fold approach was taken to the problem of broomrape control in California: (a) the strictly short-range chemical approach by fumigation, and (b) a long-range approach through cultural practises, rotations and/or the application of amendments. The chemical approach proved to be the most direct and to offer the greatest promise. In the tests reported in this

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paper, the presence of broomrape in plots receiving various treatments was based entirely upon the capacity of tomatoes to effect germination of the broomrape seed and to support the parasite on to maturity.

An area of approximately 5 acres (150 feet x 1400 feet) within a known broomrape infestation was made available to the authors for experiments early in 1955. Prior to conducting any tests, 210 soil samples each containing soil from about one-third of a square foot were taken throughout the plot and planted to tomatoes in the greenhouse. One to 96 broomrape plants (mean of 22) per sample grew from 97.6 percent of the samples on the first planting of tomatoes. About the same number grew on the second planting. These findings indicated that a square foot of the soil contained an average of 132 or more viable broomrape seeds. Subsequent tests have shown that a third planting of broomrape-infested soil to tomatoes in the greenhouse may yield as many plants as the first, so undoubtedly the estimate of 132 viable seed per square foot must be considered low.

After sampling the broomrape-infested area, it was divided at right angles to the long side, into plots measuring 50 or 100 feet by 125 feet. The tomato transplants were planted by machine in rows 5 feet apart also perpendicular to the long side of the area. Irrigation water was supplied by a head ditch running the entire length of the area (1400 feet). The ditch was made on land previously fumigated with methyl bromide under polyethylene tarpaulins at the rate of 1 pound per 100 square feet. This precaution was intended to prevent the introduction of broomrape seeds into the plot with irrigation water. Water for the irrigation head ditch was supplied by underground pipes. All cultivation was done in the direction of the tomato rows. A nontreated check 13 feet wide ran the entire length of the area, cutting across one end of each plot. There were also other nontreated check plots.

The effectiveness of treatments was determined by the actual digging out and recording of all broomrape plants occurring in plots in which the treatments had reduced the numbers enough to make the practice feasible. All plants counted were dug and removed from the plots before the seed had matured.

At the time of application of the fumigants, the soil, a silty clay loam, was in seed-bed condition as to tilth and moisture. The moisture content at different times and from the soil surface to a 6-inch depth varied from as much as 12 to 18 percent as determined on a dry-weight basis. The estimated field capacity ranged from 21 to 25 percent. Rain frequently interrupted the treatment and all areas excessively wet within plots were indicated on maps. The soil temperatures at the 6-inch depth during the treatment periods varied from 12° to 15° C, increasing slightly under the tarpaulins.

APPLICATION OF METHYL BROMIDE THROUGH HEATED COPPER COILS, 1955

Methyl bromide was applied to plots 100 x 109 feet at rates of 0.5 and 1.0 pound per 100 square feet of soil. The treatments were replicated twice. The methyl bromide was applied through heated copper coils under 4-mil thick polyethylene tarpaulins, 33 x 120 feet, supported above the ground by straw-filled burlap sacks. The edges of the tarpaulins were buried about 6 inches deep in the soil. Distribution of the methyl bromide was by means of perforated polyethylene hoses evenly spaced under the tarpaulins. The tarpaulins were left in place for a minimum of 20 to 24 hours.

Counts in the two plots receiving methyl bromide at the 1 pound per 100 square foot rate were made at four times between August 1 and November 1. The total number of broomrape plants appearing in the plots was 150 and 165. These figures included 80 and 72 broomrape plants, respectively, which appeared in the edge rows of the plots, or in areas which had been puddled by rain during application of the methyl bromide. The net figures of 70 and 93 broomrape plants per plot are considered more realistic and represent a reduction in the number of broomrape plants in areas of 1/4 acre to fewer than may occur in 1 square foot of the untreated land. Growth of the tomato plants in plots receiving the methyl bromide also was vastly improved over that of the nontreated checks and, in the opinion of the authors, represented more than control of broomrape alone.

Control at the 0.5-pound methyl bromide dosage, though highly significant, was not sufficient to warrant the effort required to make detailed counts.

Vapam (sodium N-methyl dithiocarbamate dihydrate) applied at rate of 200, 400, and 600 pounds per acre also failed to control broomrape. The Vapam required for each 50 x 109-foot plot was diluted with 50 gallons of water and sprayed onto the plots, which had been flooded previously and were standing under 4 to 6 inches of water. Vapam also failed to give control when applied 6 to 8 inches deep by chisels spaced 12 inches apart at rates up to 500 pounds per acre.

CULTURAL AND AMENDMENT PROCEDURES FAILED TO CONTROL BROOMRAPE

All cultural and amendment procedures tried in 1955 failed to control broomrape. These treatments were the application of ferric sulfate to two plots each 50 x 109 feet at the rate of approximately 6 tons per acre, and the application to a single plot of 60 tons per acre of manure. The ferric sulfate changed the soil pH in the upper 9 inches from an average of 7.5 to 3.8 which, though unfavorable to tomatoes, still supported broomrape. The heavily manured plots supported a luxuriant growth of both tomatoes and broomrape.

Two plots each 50 feet x 125 feet planted to winter barley and plowed at 2-week intervals during the early summer to dry the soil failed to show control of broomrape. Drying of the soil as by summer fallow appears to hold little promise of controlling broomrape, though pot tests conducted earlier had indicated some control (17).

To test the hypothesis of Gruscevoi (6) that certain legumes, though incapable of supporting the broomrape parasite, may nevertheless stimulate its seeds to sprout and thus reduce the inoculum, two plots, each 50 feet x 125 feet, were planted to a mixture of purple vetch, broad bean, bell bean, alfalfa, and yellow clover. Broomrape appeared in abundance on tomatoes following the legumes and it is doubtful if any reduction of inoculum had been effected. In similar tests conducted in the greenhouse, O. ramosa attacked the yellow clover and grew upon it to maturity. In the field plots, however, yellow clover has been attacked only very occasionally. O. ramosa on clover in our greenhouse tests was a small, delicate, scarcely-branched plant not greatly unlike descriptions given for O. minor, a recognized parasite of the clovers. It also is of interest to note that Halsted (7), who reported O. ramosa on tomato in New Jersey in 1904 as a thick fleshy-branched shoot, also reported O. minor on clover as a thin slightly-branched shoot. These two as described by Halsted, in our judgment, may represent the same species.

FLOOD WATER MAY DEPOSIT BROOMRAPE SEED

During the 1955-56 winter the entire broomrape-infested area under study was flooded, because of frequent heavy rains, by as much as 4 to 5 feet of water for continuous periods of more than one month. A layer of silt averaging 2 to 3 inches in thickness was deposited over the area. This served to contaminate the methyl bromide plots held over from the previous season. Eight of 50 samples of the introduced silt taken from the methyl bromide and other plots of the previous season showed broomrape plants when planted to tomatoes in the greenhouse. This indicated that the silt which had been deposited by water was carrying viable broomrape seed.

APPLICATION OF METHYL BROMIDE BY THE MACLEAN CIRCULATOR

The application of methyl bromide in 1956 was by a mechanical blower and circulator developed by the N. A. Maclean Corporation of Belmont, California. Prior to application of the gas, the soil had been listed into alternate furrows and ridges, and covered with polyethylene tarpaulins supported directly on the ridges. Methyl bromide was applied to plots 122 feet x 32 feet, the area of the tarpaulins, replicated twice at rates of 0.5, 1, and 1.5 pounds per 100 square feet, respectively. Each of these dosages gave an excellent control of broomrape (Table 1) and the control carried over into the following year, showing that the mechanical circulator provided a better distribution of the methyl bromide under the tarpaulins than was achieved the previous year. It is not known to what extent contamination, as by dust, may have contributed to the broomrape infestation noted the second year.

CHISEL APPLICATION OF A METHYL-BROMIDE-KEROSENE MIXTURE, 1956

A methyl-bromide-kerosene mixture, containing equal parts by volume, was also applied to 5 plots, numbered 10A to 10E inclusive, each 16 feet x 122 feet, by tractor chisel equipment at rates of approximately 160, 160, 220, 220, and 440 pounds of actual methyl bromide per acre, respectively. The chisel spacing was 12 inches and the injection depth 6 to 8 inches. The application of 160 pounds of methyl bromide to plot 10A was in two separate 80-pound treatments applied one day apart. The soil was rotovated just prior to the second application. Plot 10B, which received the 160-pound rate, was rolled immediately following the application

Table 1. Control of broomrape following the application of methyl bromide under polyethylene tarpaulins to listed soil by Maclean Blower. Plots were 1/10 of an acre. Soil temperature was 12 to 13° C, wind velocity, 15 to 20 mph.

Dosage in pounds per 100 square feet	Total number of broomrape plants[a] appearing in each plot the year of and the year following the fumigation.			
	FIRST YEAR		SECOND YEAR	
	REP. 1	REP. 2	REP. 1	REP. 2
0.5	29	54	34	29
1.0	22	8	41	7
1.5	12	2	33	7
Nonfumigated check	Too many to count			

[a] Counts do not include broomrape plants appearing in rows bordering untreated areas.

of the methyl bromide. Plot 10C, which received the 220-pound rate, was water-sealed. Plot 10D was covered flat with a polyethylene tarpaulin, the edges of which were buried in a furrow; plot 10E was rolled. Of these five treatments which received methyl bromide at the rate of 220 pounds per acre and were covered with tarpaulins, plot 10D only was completely free from broomrape. This indicated that chisel application of methyl bromide is practicable, providing the treated soil is covered immediately with gas-impervious tarpaulins.

Other soil fumigants tested in plots replicated twice, averaging 50 feet x 109 feet, and which failed to control broomrape were: chloropicrin, Shell experimental compound F 98, carbon disulfide, Mylone, and Vapam diluted 1:1 with water. Chloropicrin was applied at rates of 3 and 4 ml per square foot (480 and 640 pounds per acre) followed, respectively, by rolling of the soil, application of a water seal, and rotovating and rolling the soil a second time after 6 days. Shell F 98 was applied at dosages up to 105 gallons per acre by tractor applicator with chisel spacings of 12 inches. In one plot injections were made crisscross at 3-, 6-, and 8-inch depths. Shell F98 was also applied as a gas to listed soil under a polyethylene tarpaulin 30 feet x 109 feet at the rate of approximately 1 pound per 100 square feet. Carbon disulfide was injected by hand injector guns 6 inches deep at 12-inch staggered spacings at rates of 300 and 600 gallons per acre. Mylone was applied as the dry powder to soil at rates of 200, 400, and 600 pounds per acre, and mixed by rotovation to a depth of 6 to 8 inches. A significant growth response was noted in the tomatoes grown in the Mylone-treated plots at the higher dosages, and Mylone effected a delay in the appearance of broomrape. The Vapam diluted 1:1 with water was applied to plots 25 x 110 feet through chisel shanks 5 inches apart at depths of 3 and 8 inches, respectively. The rate was 20 gallons of actual Vapam per acre.

An attempt was made in 1956 to bury the broomrape-infested surface foot of soil below 12 inches, as recommended by Constancis (4), by exchanging places of the first and second foot layers of the soil. This inversion brought what was essentially subsoil to the surface and buried the top soil. Though growth of the tomatoes following this inversion process was somewhat poorer than in the general area not inverted, a considerable delay in the appearance of broomrape was effected. It cannot be determined what effect the reduced growth of the tomatoes had on this apparent suppression of broomrape.

The possibility of effecting control by planting tomatoes in a deep furrow somewhat as Morettini (11) attempted with beans was also investigated. Four trenches 2 1/2 feet wide, 1 foot deep, and 120 feet long were dug and planted to tomatoes. Broomrape appeared in abundance on these, though somewhat delayed.

Dithane D-14 (19% nabam), introduced with irrigation water up to a total of 120 gallons per acre after the tomatoes were growing but prior to the emergence of broomrape, failed to control broomrape.

Dichloral urea, applied as Crag Herbicide DCU73W between tomato rows at rates of 20 and 40 pounds per acre and mixed into the soil by rotovation, failed to control broomrape. The tomato plants showed obvious injury from the treatment but recovered.

1957 EXPERIMENTS WITH COMMERCIAL WEEDFUME

The outstanding control of broomrape achieved in 1956 with the chisel application of methyl bromide in solution prompted further tests. A methyl bromide solution, available as Weedfume (approximately 70% by weight of methyl bromide), was applied to twice replicated plots by tractor equipment through chisels spaced 12 inches apart, at rates of approximately 150, 200, 250, and 350 pounds of actual methyl bromide per acre. The soil was rolled immediately after application of the Weedfume and covered for 20 to 24 hours with polyethylene tarpaulins. The tarpaulin edges were sealed in furrows. Results are shown in Table 2. An additional series of plots was fumigated with Weedfume applied in two applications on succeeding days without tarping. The soil was plowed just prior to the second application. It was considered as probable that without tarping the methyl bromide would kill the broomrape seed at soil depths greater than 2 inches but not in the surface soil. The reason for plowing after the first application of the fumigant was to turn the surface soil under so it would be exposed to the second application. A substantial control of broomrape was achieved by the split applications of Weedfume (Table 2), but it was less than that obtained by the single application covered by tarpaulins. It is also less expensive to cover the soil with tarpaulins than to apply the high rates of methyl bromide required for two applications.

Table 2. Control of broomrape following one or two chisel applications of commercial Weedfume (70% methyl bromide) to plots 30 feet x 109 feet.

Dosage in pounds actual methyl bromide per acre	Numbers of broomrape plants appearing in each plot. Rep. 1	Rep. 2
Covered with polyethylene tarpaulins		
150[a]	4	8
200	3	0
250	4	17
350	0	5
Split treatment plowed between applications		
135 + 135	heavily infested	
200 + 200	89	21
250 + 250	42	40
300 + 300	24	25
no treatment	heavily infested	

[a]In replication 2168 pounds were actually applied.

DISCUSSION AND SUMMARY

The validity of the hypothesis set down by Baker (2) that for soil-borne diseases of plants, a control based upon eradication of the parasite usually yields more effective results than one based upon suppressing it, was borne out in these studies. Suppressive measures tried against Orobanche ramosa, an obligate parasite, allowed for marginal survival and hence continued spread of the parasite.

To date O. ramosa occurs in only one rather small area of California. Soil amendments, manures, and cultural practices which frequently resulted in improved growth of the tomato host, also resulted in larger and more fruitful broomrape plants. Treatments which suppressed growth of the tomato plant likewise resulted in reduced growth of broomrape. In that conditions most favorable for the host are often most favorable for an obligate parasite, broomrape is not unlike other obligate parasites among fungi, nematodes, and perhaps even viruses. The above principle is in contrast to one applicable to non-obligate parasites, in

which instances of active parasitism are favored by environmental conditions which favor the parasite relatively more than the host, or are less unfavorable for the parasite than for the host.

Soil amendment and cultural practices which failed to control broomrape were: (a) dry fallow, (b) rotations with legumes, (c) heavy manurings, (d) lowering the soil pH, (e) planting in deep furrows, (f) calcium cyanamide applications, and (g) deep plowing, but inadequate to bury all the seeds below 12 inches.

Soil fumigants which failed to control broomrape were chloropicrin, carbon disulfide, D-D (1:3 dichloropropene, and 1:2 dichloropropane), EDB (ethylene dibromide), Shell CBP-55 (1-chloro-3-bromopropene-1), Shell experimental compound F98, Mylone (85% 3,5-dimethyl-tetrahydro-1,3-5, 2H-thiadiazine-2-thione), and Vapam (sodium N-methyl-dithiocarbamate dihydrate).

Materials applied as herbicides which failed to give control of broomrape were Crag Herbicide-1 (90% sodium 2,4-dichlorophenoxyethyl sulfate), Dithane D-14 (19% nabam) and Crag Herbicide DCU 73W (73% dichloral urea).

Methyl bromide applied by the Maclean Blower under polyethylene tarpaulins to moist, listed soil at rates above 1/2 pound per 100 square feet (220 pounds per acre) generally reduced the infestation of broomrape in 1/4-acre plots to fewer plants than occurred in 1 square foot of nonfumigated soil. Commercial Weedfume, a solution containing 70% methyl bromide, gave excellent control of broomrape when applied by tractor-mounted chisels at rates of 175 pounds of actual methyl bromide per acre, followed by covering with polyethylene tarpaulins (18).

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CONTROL OF BACTERIAL SPOT AND RIPE ROT OF PIMIENTO PEPPER[1]

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Abstract

Spraying pimiento peppers with a mixture of streptomycin and basic copper sulfate provided best overall control of bacterial spot and ripe rot in 1957. Control of bacterial spot was also obtained in 1956 and 1957 with basic (tri-basic) copper sulfate alone and with Dyrene[2]. In 1957 mixtures of streptomycin with maneb or thiram controlled bacterial spot, but Phaltan[3] plus streptomycin was ineffective against both bacterial spot and ripe rot. Phaltan used alone was effective against ripe rot. Chlorosis and reduced yields were produced by Dyrene in 1956 and 1957 and by a mixture of streptomycin and thiram in 1957.

INTRODUCTION

Approximately 20,000 acres of pimiento peppers Capsicum) are grown annually in Georgia. In southern Georgia some fields are planted from seed but most growers buy plants that have been field grown in Florida. These fields are generally sprayed several times, and if the seed has been carefully selected and chemically treated as recommended many years ago by Higgins (2), the plants should be nearly disease-free. Actually, bacterial spot due to Xanthomonas vesicatoria (Doidge) Dowson is commonly present, perhaps because of the proximity of tomato fields to the pepper plant fields. Ripe rot (Vermicularia capsici Syd.) caused an almost total crop loss on 25 acres on a central Georgia farm in 1957, apparently due to lack of seed treatment. Cercospora leaf spot (Cercospora capsici Heald & Wolf) is the third seed-borne disease and is less likely to result in serious losses than the other two, but complete defoliation was observed in one field in 1955.

Under these circumstances it has been deemed desirable to develop a spray or dust program which would prevent severe losses. Most growers would prefer to dust since they have dusters for cotton, but the experimental work reported in this paper has been done entirely with a high pressure sprayer.

MATERIALS AND METHODS

A number of fungicides and two antibiotics were field tested on pimiento pepper in 1956. In 1957 in a similar experiment mixtures of several fungicides with streptomycin were included. Also, since pepper leaves and pods are somewhat difficult to wet, the effect of adding a surfactant (wetting, spreading and sticking agent) to several of the materials was tested. As indicated in Tables 1 and 2, the same materials were used without the surfactant.

The Truhart Perfection pimiento plants were greenhouse grown in 1956 and field grown in Florida in 1957. Plants were set out approximately May 1, 2 1/2 feet apart in rows 3 1/2 feet apart. Each two-row plot consisted of 40 plants or 1/124 acre. The yield figures in Tables 1 and 2 can be converted to pounds per acre by multiplying by 31, since there were 4 replications.

Spraying was initiated July 3 in 1956 and July 2 in 1957 at approximately the time the first pods were forming. Eight or nine applications were spaced at intervals of 7 to 14 days. The tractor and sprayer used have 28 to 30 inches ground clearance to reduce mechanical injury. The spray was applied at 350 pounds pressure and at a rate of 100 to 125 gallons per acre, using three nozzles per row.

Bacterial spot was present on the foliage throughout the season both years. Visual ratings did not seem feasible so a single percentage count of diseased leaves was made on September 12, 1956 and on August 26, 1957. Since Cercospora leafspot was present only in trace amounts, no data were obtained for that disease either year.

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[1] Journal Series, Paper No. 335. Georgia Experiment Station, Experiment, Georgia.

[2] 2, 4-dichloro-6-(o-chloranilino)-s-triazine.

[3] N-trichloromethylthiophthalimide.

Table 1. Effect of spray treatments on bacterial spot and on yield of pimiento pepper - 1956.

Treatment	Rate/100 gals. (or parts per million of antibiotics)	Bacterial spot percent leaves infected	Yield[b] (lbs.)
Nabam + $ZnSO_4$	2 qts.	35	144
Zineb	2 lbs.	32	137
Captan	2 lbs.	35	149
Dyrene	1 lb.	17	100
Dyrene	2 lbs.	13	101
Omadine (Zn salt)	2 lbs.	30	163
Maneb	2 lbs.	47	156
Thiram	2 lbs.	39	162
Tribasic copper	4 lbs.	26	129
Copper zinc	4 lbs.	24	145
Copper manganese	4 lbs.	32	158
Streptomycin	200 ppm	--[a]	137
Maneb + Triton B-1956	2 lbs.	53	118
Thiram + DuPont spreader sticker	2 lbs.	49	124
Streptomycin + Triton B-1956	200 ppm	—[a]	118
Tetracycline	200 ppm	53	132
Unsprayed check		63	137

a - Since the material became caked due to absorption of moisture before use, the disease control data was considered unreliable and was omitted.

b - Total yield from 4 replicates.

There were no significant differences in the 1956 data.

Five pickings were made each year at intervals of 2 weeks, starting in August and extending into October. Total weight and weight of pods infected with ripe rot were recorded for each plot. Bacterial spot infection of pods was also recorded but it was slight and so irregularly scattered that it was felt that the foliage data better represented the control of that disease.

A stand count was made just before or after each picking in order to correct plot yields for plants missing due to southern blight, Rhizoctonia, wind or mechanical breakage and, in one instance, lightning. The loss of plants by the end of the season amounted to 22 percent in 1956 and 13 percent in 1957 for the field as a whole. Yields were corrected for missing stand by a simple proportion.

RESULTS

Table 1 shows percentages of leaves infected with bacterial spot September 12, 1956 and also total yields obtained in five pickings. Ripe rot was present in trace amounts only, with none in the unsprayed plots in 1956. There were no significant differences.

Table 2. Effect of spray treatments on control of diseases and on yield of pimiento peppers - 1957.

Treatment	Rate 100 gals.	Ripe rot percent pods infected	Bacterial spot percent leaves infected	Yield (lbs.)[b]
Nabam + $ZnSO_4$	2 qts.	5.6	45.9	190
Zineb	2 lbs.	8.5	43.7	178
Captan	2 lbs.	7.0	37.8	150
Dyrene	1 lb.	5.6	21.7	112[a]
Omadine (Mn salt)	2 lbs.	6.6	41.7	182
Phaltan	4 lbs.	1.8	45.4	153
Maneb	2 lbs.	5.4	46.8	157
Thiram	2 lbs.	8.0	54.5	148
Phaltan	2 lbs.	4.3	43.4	171
Tribasic copper sulfate (53% Cu)	4 lbs.	7.3	23.5	157
Copper manganese	4 lbs.	4.8	41.1	149
Maneb + streptomycin (100 ppm)	2 lbs.	5.0	25.9	150
Thiram + streptomycin (100 ppm)	2 lbs.	5.2	17.8	99[a]
Phaltan + streptomycin (100 ppm)	2 lbs.	6.5	40.3	184
Agrimycin 500 (contains basic copper sulfate)				
10% Cu, 10% streptomycin (180 ppm)	1 1/2 lbs.	3.7	21.5	164
35% Cu, 3% streptomycin (108 ppm)	3 lbs.	3.6	21.0	143
Maneb + Triton B-1956	2 lbs.	6.0	34.4	169
Thiram + Triton B-1956	2 lbs.	6.3	50.5	165
Copper manganese + B-1956	4 lbs.	8.1	36.8	163
Unsprayed check		7.2	70.2	160
LSD .05		NS	15.3	39

a Significantly below yield of check. Both treatments phytotoxic, showing chlorosis.
b Total yield from 4 replications.

Table 2 gives disease control and yield data for 1957. The Agri-mycin 500 in two formulations was used instead of tank mixing streptomycin and basic copper sulfate. From Table 2 it can be seen that the second formulation listed was used at 108 ppm of streptomycin, close to the 100 ppm concentration used in the tank mixtures. Also, 3 pounds of 35% copper in this Agri-mycin 500 formulation is one half the copper concentration contained in 4 pounds of tribasic copper sulfate with 53% copper. Dyrene, tribasic copper, and all the streptomycin mixtures except the one with Phaltan gave good control of bacterial spot in 1957, and the mixtures of streptomycin with copper, maneb, and thiram provided somewhat better control of ripe rot than these fungicides used alone. Again the Phaltan mixture was the exception, with poorer control of ripe rot than with the same dosage of Phaltan alone. Cox (1) obtained somewhat similar results in control of bacterial spot of bell peppers in south Florida, although his additive effect of streptomycin and copper does not show up clearly in the bacterial spot data

reported here. The ripe rot data does seem to show an additive effect with both formulations of Agri-mycin 500.

Dyrene both years and the thiram-streptomycin mixture in 1957 produced rather severe chlorosis with some defoliation and reduced yields, which were significantly below the yield of the unsprayed check in 1957. The yield with thiram and streptomycin was significantly lower than with thiram alone. No visible injury or yield reduction resulted from the other streptomycin mixtures.

The effect of adding a surfactant, Triton B-1956, was tested both years. It was thought that ripe rot control might be improved, but this did not occur. If the 1957 data were considered alone the addition of a spreader-sticker would seem to be desirable because in all three cases a small improvement in bacterial spot control and an increased yield occurred. None of these differences were significant, however, and in Table 1 it is evident that no favorable effects were obtained from the addition of a spreader-sticker in 1956. In fact, the yield effect was reversed in every case, with maneb and thiram showing about 25 percent lower yields when used with a surfactant. It should be noted that DuPont spreader-sticker was used with thiram in 1956. Also, only two of the fungicides were the same both years. This does not seem to be too important, however, as the results were quite consistent within each test for the three pairs of treatments compared.

DISCUSSION

After 2 years' of testing fungicides for control of pimiento pepper diseases, a few trends seem apparent. Most of the individual spray materials available will not control the bacterial and fungus diseases and give good yields. Either a mixture must be used or some new material not yet tested on peppers must be found. The best mixtures tried thus far appear to be the two formulations of basic copper sulfate and streptomycin, both of which are known as Agri-mycin 500.

Bacterial spot, ripe rot, or Cercospora leaf spot can cause severe losses. All these diseases can usually be controlled by a combination of seed treatment and sanitary measures, as shown by Higgins (2). It is obvious from examining various growers' fields, however, that good control of diseases is not always achieved. The plants grown in Florida are generally preferred to home grown plants but are not always free of disease. Also some growers, for various reasons, desire to use fields which may still harbor inoculum of one or more of the pathogens. Under any circumstances where diseases controllable by spraying may be expected, a spraying or dusting program may be helpful. At present, however, on many farms the benefits resulting from spraying might not justify the cost. Most growers with small pimiento acreages will not want to purchase expensive equipment unless it can be used for other crops. No cost studies have been made, but in the experiments reported here eight spray applications appeared to be a minimum number. One of the better treatments, maneb plus streptomycin, was applied in a reduced schedule of five applications in 1957 (not shown in Table 2). No control of either bacterial spot or ripe rot was obtained. The cost of the minimum eight spray schedule could hardly be less than $25.00 per acre. At a price of $90.00 per ton and a yield of 2 tons per acre of marketable peppers from an unsprayed field (above the State average) an increased yield of 14 percent in healthy peppers would be necessary just to offset the cost of spraying. One treatment, Omadine, used in the 1956 and 1957 field tests has resulted in yields 19 percent and 14 percent higher than the unsprayed checks. Disease control was not outstanding, however. Further field tests and actual cost studies will be necessary before any program of spraying or dusting can be recommended to growers.

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RESISTANCE OF BROCCOLI AND OTHER CRUCIFERS TO DOWNY MILDEW[1]

John J. Natti

Summary

Inoculation of cotyledons provided a rapid method for distinguishing crucifer seedlings highly resistant to downy mildew from moderately resistant and susceptible seedlings in greenhouse tests. Inoculation of the foliage of crucifer plants at the three- to four-leaf stage of growth provided an effective method for distinguishing moderately resistant plants from susceptible. Five collections of Peronospora parasitica from naturally infected broccoli plants obtained from different locations in New York State appeared to be of one physiologic race. All commercial varieties of Brassica oleracea, with the exception of var. fimbriata (kale), were susceptible to this race whereas all other horticultural crucifers tested were either resistant or immune. Among plant introductions of broccoli tested for downy mildew resistance, one selection from P.I. 189028 was moderately resistant and all plants of P.I. 231210 were highly resistant. Resistant seedlings were found among the following cauliflower introductions: P.I. 208474, 208478, 208482, 209751, 209754, 209756, and 222971. In field tests, differences in resistance to downy mildew were observed among commercial varieties of broccoli but no variety was highly resistant.

INTRODUCTION

Downy mildew incited by Peronospora parasitica (Pers.) ex Fr. is one of the important diseases of broccoli, Brassica oleracea var. botrytis L., in western New York State. This disease causes leaf injury and defoliation of plants in the seedbed and the field. Severe foliage infection usually results in reduction in yields. The most serious aspect of this disease, particularly to the commercial broccoli packer, is the discoloration of the interior of the apical portion of the stem and of the stalks of the head as the result of invasion of these tissues by the fungus. Because of the hidden nature of the infection, diseased heads frequently are not detected until the crop is being prepared for processing. The presence of infected heads, even though they usually occur in low percentage of the total plant population, necessitates the careful inspection of all heads during preparation for processing. Such inspection slows down the sorting operations and increases the processing costs. All commercial broccoli varieties are susceptible to downy mildew. Control by fungicide applications has not been sufficiently effective to insure freedom from head infection (1, 2). The incidence of downy mildew has been observed to be increased as the result of the ready wetting of broccoli foliage sprayed with insecticide formulations which remove the bloom from the leaves (1). The only effective control method appears to be the development of resistant varieties. This report contains a description of methods used to test plants for downy mildew resistance in the greenhouse and the results of such tests on the resistance of broccoli and other crucifers. The results of field tests on mildew resistance of commercial broccoli varieties are included.

METHODS

Greenhouse Tests

Horticultural species among the Cruciferae were inoculated with each of 5 collections of Peronospora parasitica obtained from naturally infected broccoli plants from various locations in New York State. The uniform reaction of the crucifer species to the five cultures indicated that one physiologic race was involved in the natural infection of broccoli. One collection was maintained as a source of inoculum for all subsequent greenhouse tests. This culture was main-

[1] Approved by the Director for publication as Journal Paper No. 1113, New York State Agricultural Experiment Station, Geneva, New York.

tained by inoculating fresh broccoli plants every 5 to 7 days.

Seedlings of commercial species of crucifers and of plant introductions obtained from the Regional Plant Introduction Station, Geneva, New York were tested. The broccoli variety Waltham 29 was used as a susceptible standard in all tests. Fifty to 100 seeds of each entry were planted in 4-inch pots containing composted soil. In most instances, if the cotyledons were to be inoculated, the seedlings were not transplanted but were inoculated 7 days after emergence. In other tests seedlings were transplanted 5 days after emergence and inoculated 7 days later. For foliage inoculations, seedlings were transplanted to 3-inch pots 5 days after emergence and inoculated 18 to 25 days later. Seedlings which were selected as possible sources of resistance to be used in a breeding program were subjected to two additional inoculations at 3-week intervals.

Plants of the broccoli variety Waltham 29 with six to eight leaves were used as inoculum source plants for the greenhouse tests. These plants were atomized with a spore suspension and held in a saturated atmosphere at 65° to 70° F for 5 to 7 days. The under surfaces of the lower leaves, at this time, were covered with a dense growth of conidiophores bearing abundant spores. The spores were removed by immersing detached leaves in distilled water and brushing the spores free. The resulting suspension was filtered through two thicknesses of cheesecloth and the spore concentration was adjusted to a standard of 100,000 spores per ml. The inoculum was used within 1/2 hour of preparation.

Test seedlings were inoculated in a moist chamber by atomizing them with a spore suspension until droplets formed but did not run off the cotyledons or leaves. No special efforts were made to inoculate the underside of the foliage. When the number of plants to be inoculated was small, a DeVilbiss atomizer with bulb syringe was used. When large numbers of plants were involved, they were atomized with a paint spray gun operating at an air pressure of 10 PSI. The chamber was then humidified and the inoculated seedlings maintained in a saturated atmosphere at 65° to 70° F from 3 to 6 days, depending upon symptom development in the controls.

At temperatures of 80° F and above, sporulation is sparse or absent. Under these conditions, it may be difficult to distinguish between susceptible seedlings and highly resistant seedlings which give a fleck reaction. The same difficulty may be encountered when conidiophores and spores are washed from the foliage by droplets of water from the nozzles of the humidifying equipment or from any other source. Desiccation of the conidiophores and spores from inadequate humidification may also cause confusion in evaluating seedlings for resistance.

The foliage of broccoli plants exposed to high humidity for 10 days or more frequently became infected by an Alternaria sp. This fungus sporulated in leaf lesions. Mildew inoculum contaminated with the Alternaria spores must be discarded since the contaminant is pathogenic to crucifer seedlings. At temperatures of 65° to 70° F, on broccoli plants atomized with inoculum containing both mildew and Alternaria spores, the mildew fungus sporulated 2 to 3 days earlier than did the Alternaria sp. Mildew cultures on broccoli were maintained free of this contaminate by inoculating fresh plants every 5 to 7 days and discarding the plants from which the spores for inoculum were obtained.

Field Tests

Commercial broccoli varieties were observed for resistance to downy mildew in replicated plots in field plantings in 1956 and 1957. The plants were started in the greenhouse and transplanted to the field plots during the last week of July. The plantings were sprayed periodically with insecticides but no fungicides for control of downy mildew were applied. All infections originated from natural inoculations. In October, the severity of downy mildew infection of each plant was recorded according to the following scale: 1 = no infection; 2 = small lesions on lower leaves only; 3 = small lesions on both upper and lower leaves; 4 = small lesions on upper leaves, large lesions (1/4 inch or more in diameter) on lower leaves; 5 = large lesions on both upper and lower leaves; and 6 = leaves dead or plant defoliated except for the uppermost leaves.

RESULTS

Greenhouse Tests

The host range of the physiologic race of Peronospora parasitica obtained from naturally infected broccoli plants from various locations in New York State was restricted to varieties of Brassica oleracea (Table 1). All horticultural varieties of B. oleracea tested, with the exception of var. fimbriata (kale), were susceptible. Chinese cabbage, mustard, rape, turnip and Matthiola incana L. gave a fleck reaction with no sporulation. Radish and the other species tested were immune. Broccoli was not susceptible to a physiologic race of the mildew fungus obtained from naturally infected radish and turnip plants.

Inoculation of seedlings at the stage of growth at which the first leaf was unfolding produced the most rapid development of symptoms. With plants of this age, a dense growth of conidiophores bearing spores developed on the lower surfaces of the cotyledons of susceptible plants 3 to 4 days after inoculation. An infection characterized by dark streaks with sparse or no sporulation occurred on the cotyledons of plants with moderate resistance. A fleck reaction with no sporulation occurred on cotyledons of highly resistant seedlings.

When moderately resistant seedlings were inoculated in the cotyledonary stage and maintained in a saturated atmosphere for 5 to 6 days after inoculation, sparse to moderate sporulation occurred on the cotyledons. Under these conditions, moderately resistant seedlings were difficult to distinguish from susceptible seedlings. Inoculation of the three- to four-leaf stage provided the best method for detecting plants with moderate resistance. Foliage symptoms, although of the same type as the cotyledon symptoms, appeared 1 or 2 days later and were less severe. With seedlings possessing moderate resistance, sporulation occurred on the cotyledons but was either absent or sparse on the leaves even though scattered lesions were present. Under the same conditions, heavy sporulation occurred on the cotyledons and lower leaves of susceptible plants.

Most of the Brassica oleracea varieties obtained from the Regional Plant Introduction Station were susceptible (Table 2). Among the broccoli varieties tested, a selection from P.I. 180928 was moderately resistant. This plant was selfed and about 60 percent of the seedlings were resistant. A number of resistant plants were found among the progeny of crosses in which resistant plants were used as the male parent and susceptible commercial broccoli varieties as the female. The mode of inheritance of this resistance has not been established.

Seedlings of another broccoli introduction, P.I. 231210, appeared highly resistant (Table 2). The cotyledons of 9 of 58 plants developed small lesions with sparse sporulation while the remainder of the plants were completely free from symptoms. This introduction has been crossed with commercial broccoli varieties but seedlings of these crosses have not been tested for resistance.

Thirty-nine cauliflower introductions were tested for resistance by means of the cotyledon inoculation technique (Table 2). Resistant plants were found among the following introductions (number resistant plants = numerator; number plants tested = denominator): P.I. 208474 (8/27), 208478 (44/150), 208482 (25/81), 209751 (6/137), 209754 (29/114), 209756 (50/156), and 229751 (4/67).

Seedlings of some introductions labelled as varieties of Brassica oleracea developed a fleck reaction but leaf and hypocotyl characteristics indicated that these introductions probably were of another species (Table 2).

Field Tests

All varieties of broccoli included in these tests were susceptible to downy mildew. In the 1957 tests, the mildew-index of a strain of Italian Green Sprouting broccoli obtained from Northrup, King & Co. was significantly less than that of any of the other varieties (Table 3). In 1956, this variety had a lower mildew-index than that of all but the latest maturing varieties. Green Mountain was the most susceptible and also the earliest maturing variety. Early maturing varieties, with the exception of the Italian Green Sprouting strain mentioned above, were more susceptible to mildew than late maturing varieties. Differences in severity of mildew infection were observed among plants within a variety. This observation suggests the possibility that resistant varieties may be developed by selection from existing broccoli varieties.

Table 1. Reaction among horticultural species of Cruciferae to downy mildew in greenhouse tests.[a]

Species		Commercial Varieties
	Susceptible[b]	
Brassica oleracea		
var. acephala DC.	collard	Georgia Southern
var. botrytis L.	broccoli	Calabrese, DeCicco, Early Green Sprouting, Grand Central, Green Mountain, Midway, Texas 107, Waltham 29
var. botrytis L.	cauliflower	Early Snowball, Imp. Holland Erfurt, Purple Head, Seneca Snowball, Snowcap, Snowdrift, Snowball Imperial, Snowball Perfected
var. capitata L.	cabbage	Brunswick, Ditmarsker, Empire Danish, Golden Acre, Jersey Queen, Late Flat Dutch, Mammoth Rock Red, Marion Market, Racine Market, Reed's Glory, Res. Detroit, Wisc. All Seasons, Wisc. Hollander
var. gemmifera DC.	Brussels sprouts	Catskill
var. gongylodes L.	kohlrabi	Early White Vienna, Early Purple Vienna
var. napobrassica L.	rutabaga	American Purple Top, Imp. Long Island
	Resistant[c]	
B. oleracea		
var. fimbriata Mill.	kale	Dwarf Green Scotch, Dwarf Scotch Curled, Dwarf Blue Curled, Blue Scotch Dwarf Curled, Siberian
Brassica campestris L.	mustard	Giant Southern Curled, Long Smooth Leaf
Brassica napus L.	rape	Dwarf Essex
Brassica pe-tsai Bailey	Chinese cabbage	Chihli
Brassica rapa L.	turnip	Black Spanish, Purple Top Milan, Snowball
Matthiola incana R. Br.	stock	Ten Weeks
	Immune	
Cheiranthus allionii Hort.	wallflower	Siberian Wallflower
Hesperis matronalis L.	dame's violet	Dame's Violet
Lepidium sativum L.	cress	Fine Curled
Lobularia maritima L.	alyssum	Violet Queen
Raphanus sativus L.	radish	Cavalier, Comet, Early Scarlet Globe French Breakfast, Long Scarlet, Rosy Gem, Sparkler

a Reaction to each of five collections of Peronospora parasitica from naturally infected broccoli plants was identical.

b Susceptible seedlings developed dense growth of conidiophores and spores on cotyledons.

c Resistant seedlings gave fleck reaction with no sporulation.

Table 2. Reaction of crucifer introductions to downy mildew in greenhouse table[a]

Species	Plant Introduction Number[b]
Susceptible[c]	
Brassica oleracea var. botrytis L. (broccoli)	212592, 234599
B. oleracea var. botrytis L. (cauliflower)	163485, 164496, 164553, 165488, 173845, 205990, 205993, 205994, 206942, 208475, 208476, 208477, 208479, 208480, 208484, 208485, 209752, 209753, 209755, 209757, 209758, 212080, 212972, 213451, 217934, 225852, 225853, 226504, 231207, 231208, 231209, 232070
B. oleracea var. capitata L. (cabbage)	182148, 182150, 187232, 194069, 197040, 199950B, 211581, 211907, 212080, 220859, 227012, 229470, 230722, 232071, 235041, 235042, 235043, 235044, 235045, 235046, 236256, 236257, 236258, 236259
B. oleracea var. gemmifera L. (Brussels sprouts)	209942, BS-2
B. oleracea var. gongylodes L. (kohlrabi)	211908, 231047, 227232, 188611
Brassica sp. (probably oleracea variety)	220646, 223704
(Group I) Resistant[d]	
Brassica oleracea var. botrytis L. (broccoli)	189028, 231210
B. oleracea var. botrytis L. (cauliflower)	208474, 208478, 208482, 209751, 209754, 209756, 222971
(Group II) Resistant[e]	
Brassica carinata	199947, 231046
Brassica sp.	199949, 204682, 204683, 207465, 211000, 212083, 212974, 220282, 220511, 220512, 220573, 195554
Brassica oleracea (probably not oleracea)	115881, 115882, 234600, 234601, 231211

a Fifty to 100 seedlings of each introduction inoculated with spores from broccoli plant infected with downy mildew.

b Plant Introductions obtained from the Regional Plant Introduction Station, Geneva, N. Y.

c Susceptible: All seedlings were severely infected; heavy sporulation on cotyledons and leaves.

d Resistant (Group I) = accessions contain resistant seedlings; resistant plants developed dark streaks in cotyledons and leaves. Sporulation sparse or absent. P.I. 231210 also contained immune seedlings.

e Resistant (Group II) = All plants gave fleck reaction with no sporulation.

Table 3. Severity of downy mildew infection (Mildew-index) of broccoli varieties in field tests in 1956 and 1957.

Variety	Source of seed	Days to harvest[a]	Mildew-index[b] 1956[c]	1957[d]
Green Mountain	Ferry-Morse Seed Co.	50	5.2	4.9
Texas 107	Assoc. Seed Growers, Inc.	55	3.7	4.7
Italian Green Sprouting	Northrup, King and Co.	60	2.4	2.9
Extra Early Green Sprouting	Corneli Seed Co.	60	3.4	4.4
DeCicco	Assoc. Seed Growers, Inc.	70	3.7	4.3
E-1 A	Eastern States Farmers' Exchange	70	3.6	---
E-1 B	Eastern States Farmers' Exchange	75	3.3	---
Green Sprouting T-strain	Ferry-Morse Seed, Co.	75	4.6	4.2
Green Sprouting Early	Corneli Seed Co.	75	4.7	---
Chinook	Woodruff	78	---	3.5
K and V	Assoc. Seed Growers, Inc.	78	4.9	4.0
Midway	Assoc. Seed Growers, Inc.	80	3.3	4.0
Early Strain	Ferry-Morse Seed, Co.	85	4.9	4.4
Early Green Sprouting	Corneli Seed Co.	85	---	4.3
Waltham 29	Assoc. Seed Growers, Inc.	90	3.6	3.8
Grand Central	Assoc. Seed Growers, Inc.	98	2.5	3.8
Medium	Assoc. Seed Growers, Inc.	110	2.3	3.6
Green Sprouting- Medium	Corneli Seed Co.	110	2.1	3.4
Least Difference for Significance	19:1		0.4	0.4
	99:1		0.5	0.5

a Days from date plants were set in the field to date at which 75 percent of the plants had produced marketable heads.

b Mildew-index is the severity of downy mildew infection (per plant basis) according to the following scale: 1 = no infection; 2 = small lesions on lower leaves only; 3 = small lesions on both upper and lower leaves; 4 = small lesions on upper leaves, large lesions (1/4-inch or more in diameter) on lower leaves; 5 = large lesions on both upper and lower leaves; and 6 = leaves dead or plant defoliated except for uppermost leaves.

c In 1956, plantings consisted of 6 replications of 16 plants each; plants were scored for mildew on October 22.

d In 1957, plantings consisted of 4 replications of 25 plants each; plants were scored for mildew on October 8.

DISCUSSION

The physiologic race of Peronospora parasitica obtained from naturally infected broccoli plants was pathogenic only to varieties of Brassica oleracea. The other crucifer species tested cannot be considered to be of any importance as sources of inoculum or as overwintering hosts for the race of the mildew fungus attacking broccoli. These results suggest that cruciferous weeds do not serve as sources of inoculum. Since susceptible varieties of B. oleracea do not normally survive the winter in this region, the mildew fungus attacking broccoli most probably survives the winter in the form of oospores.

The cotyledon inoculation method for testing crucifers for downy mildew resistance is rapid and permits the testing of a large number of seedlings at one time. Seedlings do not require transplanting, can be inoculated 4 to 5 days after emergence, and examined for mildew infection 3 to 4 days later. The cotyledons are so susceptible that there is little possibility for plants to escape infection. Only very rarely have plants selected as resistant by the cotyledon inoculation method been found to be susceptible in subsequent tests. The cotyledon inoculation method is ideal for distinguishing between very resistant and susceptible seedlings but may be too severe a test to detect seedlings which possess moderate resistance. This method of inoculation, therefore, should not be used to evaluate breeding lines which have moderate resistance. Such breeding lines can be tested by inoculating the foliage of seedlings.

Progeny of crosses in which the broccoli P. I. 189028 is the source of mildew resistance should be tested by the foliage inoculation method. Sporulation occurs on cotyledons of this introduction so that it may not be possible to observe clear-cut differences between resistant and susceptible seedlings when the cotyledon inoculation method is used. However, when the foliage inoculation method is used, scattered lesions may be present in leaves of the resistant plants; whereas under the same conditions, the under surface of the foliage of susceptible plants will be covered with a dense growth of conidiophores.

Seedlings of P. I. 231210 are highly resistant. This introduction resembles purple cauliflower and has been successfully crossed with the broccoli variety Waltham 29. The cotyledon inoculation method of testing seedlings can be used effectively with progeny of this introduction.

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ROLE OF DEAD FLOWER PARTS IN INFECTION OF CERTAIN CRUCIFERS BY SCLEROTINIA SCLEROTIORUM (LIB.) D BY.[1]

D. M. Mc Lean[2]

Abstract

The importance of dead flower parts at the loci of infection by ascospores of Sclerotinia sclerotiorum on broccoli, cauliflower, and cabbage is emphasized. Foliar infections by mycelium from mats on potato dextrose agar or from sclerotia occurred only through abrasions or when dried broccoli flower parts were present on the leaves. Cabbage flowers in situ were infected by ascospores.

INTRODUCTION

Although it is reported that infections by Sclerotinia minor occurred after placement of mycelial mats on lettuce leaves (5), it has been demonstrated that this organism cannot invade the unwounded epidermis of lettuce (1, 9). Several investigators have pointed out that mycelial infections of lettuce by S. minor and of a number of vegetables by S. sclerotiorum occur at the soil line (4, 5, 6, 8, 9). The importance of non-living organic matter as an intermediary between the soil-borne mycelium and the locus of infection in lettuce was emphasized by Purdy and Grogan (9).

Above-ground infections are initiated by ascospores, and a number of workers have reported spore infections through abrasions or directly (4, 8). Despite these reports, it is generally concluded that germ tubes do not infect tissues directly (3) but do so mostly through the intermediary of dead or senescent blossom parts (2, 3, 4, 7, 8, 10, 11). Moore[3], after observing Sclerotinia sclerotiorum on many crop plants in Florida, made the following remark: "My tests over a long period of time together with literally hundreds of field observations clearly indicate that spore infection by this organism is limited to the interposition of dead organic matter. I have never seen one case where I felt that the infection began through direct mycelial penetration of clean, freshly growing tissue. This has been true in this locality with respect to all crops grown, and among these I would list beans, potatoes, tomatoes, squash, cucumbers, peppers, lettuce, celery; in fact, everything that has shown the disease has demonstrated this one fundamental fact in so far as infection is concerned."

Studies were made on the relation of dead flower parts to infection of broccoli, cauliflower, and cabbage by mycelium and ascospores of S. sclerotiorum in attempts to correlate such infections with a serious stalk rot of certain cruciferous seed crop plants in northwestern Washington. The relation of Sclerotinia stalk infections to foliar infections resulting at the loci of deposition of flower parts following pollination was pointed out previously (7). The results reported herein re-emphasize the importance of non-living organic matter in infection cycles by this organism.

EXPERIMENTATION AND RESULTS

Five healthy headed potted cabbage plants, Copenhagen Market type, were treated in each of the following manners: 1) a mycelial mat of S. sclerotiorum on potato dextrose agar was placed on unabraded tissue in a leaf axil; 2) a similar mycelial mat was placed in a leaf axil after punctures were made with a sterile needle; and 3) a mycelial mat was placed over dried broccoli flower parts in a leaf axil or on a concave leaf area. Flowers were obtained from commercial broccoli the previous fall and dried. As checks, needle punctures were made on the foliage and broccoli flowers were placed on similar healthy plants without the fungus. Following the treatments, the plants were placed in a greenhouse humidifier for 8 days.

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[1]Scientific Paper No. 1703, Washington Agricultural Experiment Stations.

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The procedures just described were duplicated by using as inocula sclerotia obtained from cabbage seed cleanings instead of mycelial mats. The results (Table 1) demonstrate that the fungus mycelium did not invade through unwounded tissue but only through injuries or at the loci of dead flower parts.

In a separate experiment, 12 potted plants each of broccoli, cauliflower, and cabbage in the 10- to 12-leaf stage were placed in the greenhouse humidifier at an average temperature of 18° C. Several mature apothecia, attached to sclerotia, were placed on the surface of moist sand in small pots and set at random in the humidifier. A forced fog circulated uniformly in the chamber for 45 seconds at 15-minute intervals. Dried broccoli blossom parts were distributed on the foliage of half the plants of each species. The inoculated plants remained in the humidifier for 6 days, and then were transferred to a greenhouse bench. Infections occurred on plants only at the loci of the dead flower parts, but no infections occurred in the absence of flower parts (Figure 1, A, B, and Table 1).

All experiments were repeated several times with similar results.

To determine whether blossoms might become infected before they fall, cabbage inflorescences with mature open blossoms were placed in the humidifier where they were exposed to ascospores dispersed from apothecia. After 2 days, senescent petals appeared grey with white fungus hyphae on the surface. Sclerotinia sclerotiorum was consistently established on potato dextrose agar in laboratory cultures from these infected blossoms, as well as in those from foliar lesions (Figure 2).

Table 1. Infection of broccoli, cauliflower, and cabbage by Sclerotinia sclerotiorum in relation to injury and presence of parts of dead broccoli flowers.

Inoculum and tissue treatment	Plants in each test	Infection centers* on Broccoli	Cauliflower	Cabbage
	Number	Number	Number	Number
Mycelium:				
Tissue wounded	5	5	6	6
Tissue with flower parts	5	7	9	7
Tissue not wounded	5	0	0	0
Sclerotia:				
Tissue wounded	5	4	3	3
Tissue with flower parts	5	4	5	4
Tissue not wounded	5	0	0	0
None:				
Tissue wounded	5	0	0	0
Tissue with flower parts	5	0	0	0
Ascospores:				
Tissue with flower parts	6	9	10	12
Tissue not wounded	6	0	0	0

*Averages for 3 replications. Some infected plants were killed.

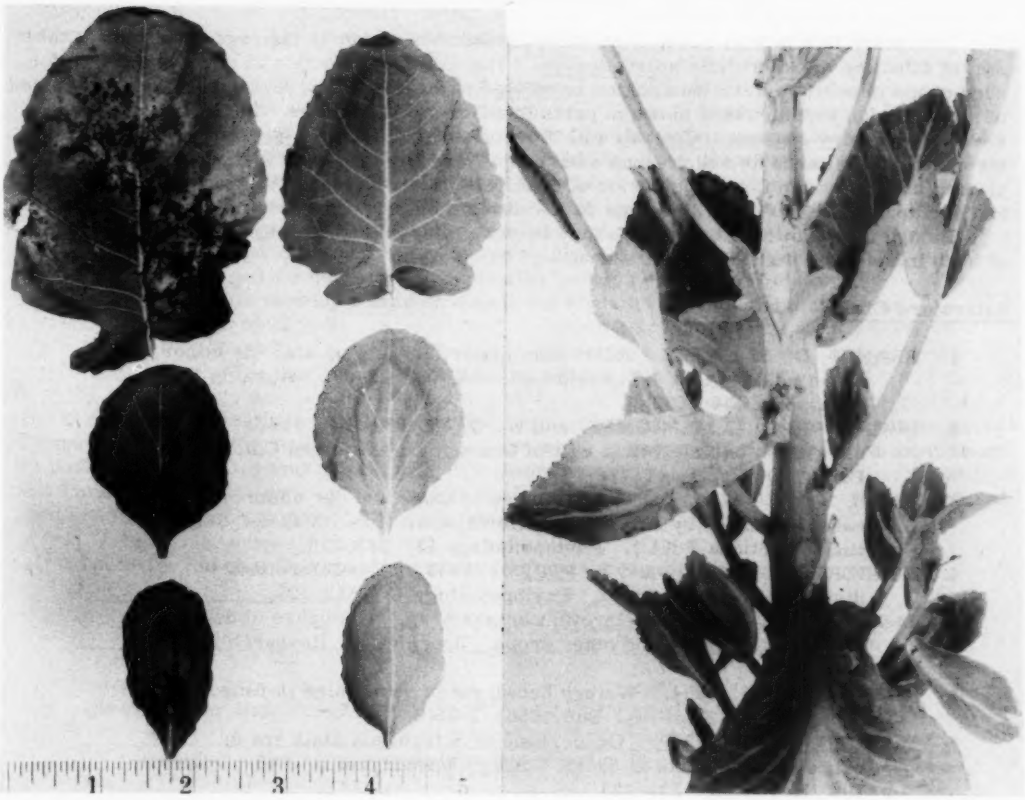

FIGURE 1. A, upper to lower: broccoli, cauliflower, and cabbage leaves (left) showing infections by Sclerotinia sclerotiorum at the loci of deposition of dried broccoli flowers after inoculation with ascospores; healthy leaves (right). B, a cabbage seed plant taken from the field showing the natural relation to ascospore infections and deposition of flower parts.

FIGURE 2. Upper: Isolate of Sclerotinia sclerotiorum from foliage of test plant after ascospore infection at loci of dried broccoli flower parts. Lower: Culture of the same fungus from cabbage blossoms exposed to ascospores in a humidifier.

DISCUSSION

Literature is rife with information showing the role of non-living organic matter at the loci of infection by Sclerotinia sclerotiorum. The extent of infection in crucifers grown for seed crops in northwestern Washington is related to the frequency during the flowering period of precipitation which causes blossom parts to adhere to the foliage. Infections are less severe during dry seasons. Commercial broccoli and cauliflower, grown for processing in the same general area as cruciferous seed crops, are not affected. Although these are not planted until after the flowering period of crucifers grown for seed, they are often irrigated and apothecia are present. Blossoms do not develop on crucifers grown for processing.

Though it is shown that blossoms may become infected before they fall, the importance of such infection in the field is not known.

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ONION FUSARIUM BASAL ROT IN ARIZONA[1]

Robert B. Marlatt[2]

Abstract

Fusarium basal rot of onion, caused by Fusarium oxysporum f. cepae, was found during the latter part of May 1956 near Tempe, Arizona. Onion varieties L 690 and Granex had significantly less basal rot than Eclipse or Texas Hybrid 28 varieties. A greenhouse experiment showed that onion bulbs were readily infected by the fungus when roots had been injured mechanically. A storage experiment revealed that apparently healthy bulbs, grown in inoculated soil, could develop Fusarium basal rot when stored for 3 months at 75° to 85° F.

VARIETAL REACTION

In the latter part of May 1956, Fusarium basal rot caused a serious loss of onions near Tempe, Arizona. The decayed bulbs were found while harvesting the plants from experimental plots located in a commercial field. Symptoms were similar to those described by Walker and Tims (3). The incidence of decayed bulbs was recorded for six onion varieties which had been randomized and replicated 7 times. Varietal reactions are shown in Table 1.

Table 1. Reactions of onion varieties to Fusarium basal rot.

Variety :	L 690	Granex	L 365	Texas Hybrid 23	Eclipse	Texas Eclipse Hybrid 28
Rotted bulbs: (Average Per Rep.) :	1.43[a]	1.57[ab]	4.57[abc]	4.86[bc]	5.29[c]	5.71[c]

[abc] Figures with the same superscript are not significantly different at the 1% level, Duncan's multiple-range test.

Varieties L 690 and Granex had significantly less basal rot than Eclipse or Texas Hybrid 28.

PATHOGENICITY

In order to be certain that the disease was Fusarium basal rot, an isolate of Fusarium oxysporum from decayed bulbs was tested for pathogenicity in the greenhouse.

Texas Hybrid 28 seeds were surface sterilized and sown in flats of pasteurized soil. Plants were transplanted when they were a month old to 24 pots of pasteurized soil and 24 containing soil that had not been steamed.

Unpasteurized soil was used to distinguish a weak parasite, which might infect onions in the absence of competing organisms, from a pathogen which could cause disease under field conditions. Half of each lot of 24 pots was inoculated with the Fusarium which had been grown in Czapek-Dox broth and macerated in a Waring Blendor. Some soil and roots near each plant were cut with a spatula and the inoculum poured into the crack at monthly intervals during the 5-month growing period.

When bulbs had matured and all foliage had dried, one plant in each pot was randomly selected and rated for basal rot. Disease severity was rated as 0 to 10, 0 being no decay and 10 representing a completely rotted bulb.

Bulbs grown in inoculated soil showed a total disease rating of 33, which included a 12 rating from pasteurized soil and 21 from unpasteurized. No basal rot was found during harvest

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of bulbs from soil which had not been inoculated. There were no symptoms of pink root disease, which Davis and Henderson (1) reported to be commonly associated with Fusarium basal rot. Perhaps infection was induced by root injury during soil inoculation.

STORAGE

After being given a disease rating, each bulb was placed in a paper bag and stored at 75° to 85° F for 3 months. At the end of the storage period, bulbs grown in inoculated soil exhibited a total disease rating of 77, whereas those grown without inoculum still had no Fusarium basal rot. As reported by Link and Bailey (2), basal-rot symptoms were manifested during storage of bulbs which had appeared healthy when they were harvested.

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ARIZONA AGRICULTURAL EXPERIMENT STATION, MESA

ZINEB AS A PROTECTIVE FUNGICIDE FOR THE CONTROL OF ASPARAGUS RUST

M. B. Linn and K. R. Lubani[1]

Summary

Zineb (65%) as a spray gave better control of asparagus rust in Northern Illinois than did maneb, ferbam or captan. A schedule of eight applications at about 8-day intervals beginning on June 18 was superior to four or six applications. Zineb is recommended at 2 to 3 pounds in 100 gallons of water per acre, beginning shortly after harvest ceases and continuing at 8- to 10- day intervals until around August 15.

INTRODUCTION

The use of protective fungicides to control the uredial and telial stages of asparagus rust, caused by Puccinia asparagi DC., has been investigated by several workers, beginning in 1898 with Halsted (2) who reported on experiments with various formulations of bordeaux mixture. Several other copper fungicides as well as potassium sulfide and permanganate have been tested (4, 5, 8, 9) but all have proven to be relatively ineffective. Sulfur dust was employed by Smith (6, 7) with some success. Although sulfur has been recommended in several States, it has not given consistently good results and has not been widely adopted by growers as a standard control measure. Kahn et al. (3) were not able to obtain satisfactory rust control with spray or dust formulations of several fungicides, alone or in combination. These chemicals included sulfur, zineb (zinc ethylenebis [dithiocarbamate]), ferbam (ferric dimethyldithiocarbamate), and Puratized Agricultural Spray (tris (2-hydroxyethyl) (phenylmercuri) ammonium lactate). More recently, Van der Vliet (10) reported that asparagus rust was effectively controlled in the Netherlands by spraying with zineb (Dithane Z-78) at a concentration of 0.2% plus an unspecified wetting agent. Approximately 100 gallons of spray were applied per acre at 10-day intervals. Ferbam (Fermate) was somewhat less effective and copper oxychloride (Kopersept), the only other fungicide in the tests, caused burning of the foliage.

This paper presents the results of 2 years' of experimentation with protective fungicides for asparagus rust control in a field of the Rochelle Asparagus Company, Rochelle, Illinois. The objective in 1955 was to determine which of four fungicides would give best control and in 1956 to determine how late in the summer a fungicide would need to be applied to give commercial control.

METHODS AND MATERIALS

In both years the experimental areas consisted of a portion of a 240-acre asparagus field of the Robert's strain, Mary Washington variety. The asparagus was planted in 1954 and was not harvested in 1955, but was cut for a 2-week period in 1956 beginning around May 10. This field was immediately east and leeward with respect to the prevailing wind of an older field with a heavy infection of rust in 1954. The experimental plots and buffer plots between treatments were four rows wide, with individual rows 4 feet apart and 1/2 mile long, with plants 18 inches apart in the row. The treatments were not replicated.

The sprayer was a three-wheel, high-clearance machine equipped with a boom designed to cover two rows with five cone-type, Teejet nozzles[2] to each row. Two nozzles were set to cover each side of the row and one was directed downward to cover the top of the plants. Each fungicide was applied in 100 gallons of water to the acre at 135 pounds pressure in 1955,

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[1]Professor and Assistant, respectively.

This research was supported in part by funds from a cooperative project with the Department of Horticulture, University of Illinois. Acknowledgment is made of the assistance of Mr. Ralph Castensen of the Rochelle Asparagus Company, Rochelle, Illinois, and Dr. A. E. Thompson, Department of Horticulture, University of Illinois. Dr. W. C. Jacob, Department of Agronomy, advised in statistical analyses of the experimental data.

[2]No. 1/4 TT D4-45 with a maximum capacity of 1.11 gallons per minute per nozzle.

and at 85 pounds in 1956.

Fungicides used in 1955 were captan (Orthocide 50W -- N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), zineb (dry Parzate), maneb (Manzate -- manganous ethylenebis [dithiocarbamate]), and ferbam (Fermate). Zineb (dry Parzate) was the only fungicide used in 1956.

In 1955 the first applications were made on May 20, 12 days after emergence of the first spears and approximately 10 days before the first appearance of urediospores. Eleven applications were made at an average interval of 9 days with the last on August 18. Data on severity of rust were taken on September 9.

In 1956 zineb was applied beginning on June 18, which was approximately 25 days following harvest and about 5 days after the first appearance of urediospores. Subsequent applications were made at about 8-day intervals. In one plot, treatment was discontinued on July 14 after four applications, in a second on July 30 after six applications, and in a third on August 13 after eight applications. Data on severity of rust were recorded on August 28.

In both years, the efficacy of the various treatments was determined by estimating the severity of rust infection according to the numerical scale, developed by Kahn et al., of 0, 2, 4, 6, 8, 10, where 0 = no infection and 10 = severe infection. In 1955, 50 individual stalks at a distance of 100 to 300 feet from the ends of each plot were evaluated in this fashion. In 1956, 25 stalks were classified from each end and from the center of each plot. In both years, data were taken on the two inside rows of each four-row plot.

Climatological conditions from May through September in both years were conducive to the development of asparagus rust. Monthly mean temperatures for the 2 years ranged from a low of 57° F in May of 1955, to a high of 74° in July of 1955. Beraha (1) found that temperatures between 77° and 85° are most favorable for the production of urediospores, 50° to 60° for germination of urediospores, and 60° to 82° for mycelial development within the asparagus plant. Rainfall was deficient during July and August of 1955, and June of 1956. However, early investigators (5, 8) have pointed out that the intensity of rust is directly proportional to the prevalence of dew, which is frequent during the growing season in Northern Illinois.

RESULTS

Zineb was superior to maneb, captan, and ferbam in the control of asparagus rust (Table 1). These data also confirm the findings of Van der Vliet with respect to the superiority of zineb over ferbam. Even though infection was more severe in the west end of the plots, that is, the end nearer the adjoining severely affected field, a relatively higher degree of control was obtained with zineb in this more severely affected end of the field.

Eight applications of zineb gave a significantly higher degree of control than did four or six treatments (Table 2). Again, somewhat better control was obtained at the west end of the field. It is likely that control would have been better had the first application been made 1 week earlier or before the initial appearance of urediospores.

Table 1. Efficacy of zineb, maneb, captan and ferbam in the control of the uredial and telial stages of the asparagus-rust pathogen, 1955.

Fungicide	Pounds[a] per acre	Mean infection indices: Location in field: West	East	Mean for treatment
Zineb	2	0.9	0.7	0.8
Maneb	1 1/2	3.8	1.4	2.6
Captan	4	5.2	3.2	4.2
Ferbam	2	5.8	3.9	4.8
None	-	6.6	4.6	5.5
L.S.D. at 0.05 level				1.7

[a]Based on the proprietary formulation.

Table 2. Control of the uredial and telial stages of asparagus rust in relation to number of applications of zineb, beginning on June 18, 1956.

Number of applications	Applications[a] ending on	Mean infection indices			
		West	Center	East	Mean for treatment
4	July 14	4.5	4.5	3.0	4.0
6	July 30	4.6	3.9	2.9	3.8
8	Aug. 13	2.9	2.3	1.7	2.3
None	---	7.4	6.6	5.8	6.6
L.S.D. at 0.05 level					0.4

[a]Rate of application = 2 pounds of 65% zineb (dry Parzate) per acre.

DISCUSSION AND CONCLUSIONS

The relative degree of control obtained by Kahn et al. with a ferbam spray was about the same as in the present investigation. They used zineb (dry Parzate) but only in a dust formulation. It is evident that lack of control in their experiments was due to an insufficient number of applications[3]. In the remaining two experiments, the level of infection was too low to permit an adequate evaluation of several fungicides. This was particularly true in one instance where a total of 16 applications of seven fungicides were made at 2- to 4-day intervals, resulting in a final average infection index of 2.0 for the fungicides and also for the control.

Any rust-control program that aids in the growing of vigorous seedlings and at the same time represses the inoculum potential for older plantings is worthwhile. Thus, a protective fungicide program with zineb will be most profitable if carried out in young, uncut fields. Primary rust infections from aeciospores ordinarily do not occur in older fields that are not harvested until around July 1. Plants that develop after July 1 are commonly infected only by wind-borne urediospores from uncut plantings.

Good control of rust with zineb applied on a commercial basis in 1957 was reported by the Rochelle Asparagus Company[4]. A 38-acre field of 1-year-old asparagus was sprayed with dry Parzate at approximately weekly intervals beginning on June 4 and ending on July 11. The dosage was 3 pounds of Parzate in 60 gallons of water to the acre, using the same sprayer employed in the 1955-56 experiments. However, Kromer flat-spray nozzles with a capacity of 0.6 gallons per minute at 100 pounds pressure were used instead of Teejet nozzles. Three nozzles were used to the row during the first half of the spraying schedule when the plants were relatively small, and five nozzles to the row thereafter. In September the sprayed asparagus was "only about 10 percent infected while the untreated asparagus was so severely infected that over 50 percent of the plants had lost their needles."

The current recommendation for control of asparagus rust in Illinois is to commence spraying with 65% zineb 2 to 3 pounds in 100 gallons of water to the acre at 100 to 200 pounds pressure, beginning in uncut fields no later than June 1. In fields that have been harvested[5] spraying should start as soon as the new plants begin to "fern out" or earlier if uredia appear. Subsequent applications should be made at 8- to 10-day intervals until August 15 or thereabouts. If the infection index is no higher than 2.0 by August 15, the losses from rust are not likely to be of any significance. Where dusts can be used more advantageously than sprays, it is suggested that an 8% to 10% zineb dust (5.2% to 6.5% active) be used at the rate of 30 to 50 pounds to the acre following the same schedule as for spraying.

[3]Three applications of dust by airplane starting June 15 and ending on July 17, 1950.
[4]From a report submitted by Mr. Ralph Castensen of the Rochelle Asparagus Co.
[5]Harvest in Illinois may end anytime between May 15 and July 1 depending largely on the age and vigor of the planting.

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RESPONSE OF PEAR AND CHERRY ROOTSTOCKS TO STREPTOMYCIN AND TETRACYCLINE WHEN APPLIED TO CONTROL CROWN GALL

Peter A. Ark[1] and David J. Bingham[2]

Summary

It can be stated that neither streptomycin nor tetracycline gave a successful control of crown gall on either pear or cherry rootstocks having the disease in incipient form, when applied as a 60-minute dip.

In some districts of the West the soil can be so heavily contaminated with crown gall bacteria, Agrobacterium tumefaciens, that the growing of budded varieties of both pear and cherry on rootstocks received from such places may be very unprofitable on account of the numerous galls developing from incipient infections on the roots.

In an attempt to prevent or minimize the occurrence of the disease on such roots two antibiotics that showed a high degree of activity, in vitro, against A. tumefaciens, were tested under field conditions on French pear and Mazzard cherry seedling rootstocks obtained from nurseries where the soil was known to be contaminated with crown gall bacteria. The roots and the tops of the seedlings were pruned by the cooperators to conform to their usual practices, tied into bundles of approximately 200 seedlings each, and dipped for 1 hour in the solutions containing 300 and 500 ppm streptomycin (agricultural grade), and 100 and 200 ppm tetracycline (agricultural grade).

To aid the penetration Triton B-1956 was added at 3 ml per gallon. The treated seedlings were planted immediately after the treatment without being rinsed in water. Untreated seedlings were planted in each row as checks alongside the treated row. The rootstocks were budded during the summer of 1956 and the yearling fruit trees were dug in the winter of 1957 at which time careful records were taken as to the presence of crown gall infections.

Table 1. Occurrence of crown gall on Bartlett pear trees on antibiotic-treated and untreated French pear rootstocks. Planted and budded in the Summer of 1956, dug in the summer of 1957.

		Rootstocks treated				Rootstocks untreated		
Test	Treatment	Trees treated	Trees infected	Percent infected	Percent control	Trees untreated	Trees infected	Percent infected
1.	Streptomycin, 500 ppm.	275	2	.73	42.9	575	10	1.7
2.	" "	258	2	.70	35.0	500	10	2.0
1.	Streptomycin, 300 ppm.	300	4	1.30	59.0	316	7	2.2
2.	" "	250	6	2.40	52.2	500	23	4.6
1.	Tetracycline, 200 ppm.	275	18	6.50	-96.9	575	19	3.3
2.	" "	250	12	4.00	58.8	305	21	6.8
1.	Tetracycline, 100 ppm	284	4	1.40	63.6	316	7	2.2
2.	" "	185	4	2.10	32.3	305	21	6.8

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Table 2. Occurrence of crown gall on Burr Hardy and Comice pear trees on antibiotic-treated and -untreated French pear rootstock. Planted and budded in the summer of 1956, dug in the winter of 1957.

Treatment	Rootstocks treated				Rootstocks untreated		
	Trees treated	Trees infected	Percent infected	Percent control	Trees untreated	Trees infected	Percent infected
Streptomycin, 500 ppm.	1002	12	1.1	44.2	486	12	2.4
Streptomycin, 300 ppm.	1004	18	1.8	33.3	605	16	2.7
Tetracycline, 200 ppm.	1000	22	2.2	21.4	633	18	2.8
Tetracycline, 100 ppm.	882	21	2.4	66.7	650	47	7.2

Table 3. Occurrence of crown gall on Royal Anne cherry trees on antibiotic-treated and -untreated rootstock. Planted in the summer of 1956, dug in the winter of 1957.

Treatment	Rootstocks treated				Rootstocks untreated		
	Trees treated	Trees infected	Percent infected	Percent control	Trees untreated	Trees infected	Percent infected
Streptomycin, 500 ppm.	467	147	31.0	-89.2	322	53	16.4
Streptomycin, 300 ppm.	536	151	28.3	-100.5	348	49	14.1
Tetracycline, 200 ppm.	482	25	5.2	29.4	318	57	17.9
Tetracycline, 100 ppm.	444	16	3.6	19.1	344	65	18.8

The experimental results (Tables 1, 2, and 3) indicate that no perfect control was obtained. There was a very pronounced difference in the response of the different rootstocks to both streptomycin and tetracycline. Most striking was an unusual abundance of galls on all streptomycin-treated Royal Anne cherry trees on Mazzard cherry rootstock. The galls appeared larger in size and more numerous than in the check. While no reasonable explanation can be offered for the failure of tetracycline antibiotic at 200 ppm to give some control of crown gall in the Bartlett pear trees, it seems that pears in general responded to both antibiotics in about the same degree with perhaps a slight tendency in favor of tetracycline. In the case of the Royal Anne cherry the tetracycline tended to give somewhat favorable results.

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TECHNIQUES FOR ISOLATING PHYTOPHTHORA SPP. WHICH ATTACK CITRUS

L. J. Klotz and T. A. DeWolfe

Easy methods have been devised for isolation of Phytophthora spp. pathogenic to citrus. P. citrophthora, P. parasitica, P. syringae, and P. hibernalis are the species found attacking citrus in California. These fungi in fruit two-thirds or more decayed can generally be recovered free of contaminating organisms by extracting and planting the seeds aseptically on potato-dextrose-agar or other suitable medium. In earlier stages of fruit rot the fungi may also be taken in tissue from the advancing margin of decay in the albedo, but contamination is more frequently encountered with this than with the seed method.

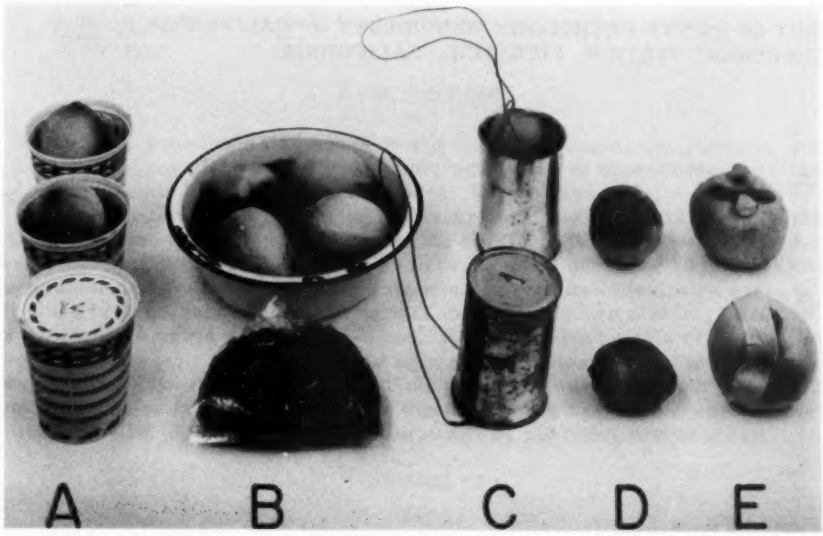

FIGURE 1. Equipment for easy isolation of Phytophthora spp. attacking citrus. A -- Paraffined paper cups (16 ounce) for collecting soil which is saturated with water, and lemons on surface for trapping zoospores. B -- Polyethylene bag for collecting soil, basin with moistened soil, and orange and lemons as zoospore trap. C -- Lemon or orange placed in can with drainage hole and wire bail. This is buried in soil under irrigation furrow, and 4 to 10 days after irrigation or rain pulled out, using top of bail protruding above soil surface. D -- Brown rot of orange and lemon resulting from infection by zoospores. E -- Apple with cores of flesh removed with steel cork borer. Infested soils or infected roots, bark, or leaves are placed in hole which is then covered with adhesive tape. Phytophthora spp. in apple tissue usually outgrow other fungi and bacteria, enabling isolation from advancing margin of decay.

Apples are useful for direct isolation from roots, bark, leaves, and soil. With forceps the infected tissue may be forcibly inserted directly into the flesh of the apple or a cylinder of the flesh may be removed with a steel cork borer and the diseased tissue or infested soil placed in the hole which is then closed with a piece of adhesive tape (Figure 1 E). In apple tissue Phytophthora spp. usually outgrow the other organisms. Pure cultures can be taken from the advancing margins of the decay. Apples may also be used for freeing agar cultures of Phytophthora from bacteria and some other organisms. However, where some Pythium, Rhizopus, or Mucor species are contaminants this method is not applicable because these fungi develop faster than Phytophthora spp.

Figure 1A shows samples of soil collected in 16-ounce wax-coated paper cups. These are transported to the laboratory where lids are removed, water added to saturate the soil, and clean, unsprayed lemons placed on the soil surface to trap the fungi. Incubation at 20°C permits growth of P. citrophthora, P. parasitica, and P. syringae and invasion of lemon or orange

fruit. Figure 1B illustrates a modification of that in Figure 1A, the soil being collected in polyethylene bags, dumped into pans, saturated with water, and citrus fruit placed on the wet surface to trap the zoospores.

Figure 1C shows a field method for trapping the fungi. A lemon or orange is placed in a can provided with a drain hole and wire bail as shown. This is buried 6 to 12 inches under the soil surface or irrigation furrows, the top of the wire bail protruding above the furrow to permit easy removal of the can with fruit. Depending upon soil temperature, 4 to 10 days following irrigation or rain the can with fruit is pulled out and isolations made from fruit showing brown rot.

In California, P. hibernalis, which grows in a lower range of temperature than the other three species, has to date been recovered only from oranges infected in the orchard.

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AN IMPROVED METHOD OF SCREENING POTENTIAL SOIL FUNGICIDES AGAINST FUSARIUM OXYSPORUM F. CUBENSE

A. G. Newhall

Summary

The percolation of a number of fungicides through a column of soil, described by Thomas (2), in which discs of a fungus are placed at different depths and later removed for viability tests, has revealed that many chemicals, even formaldehyde, are rendered ineffective on their way down through soil. Of 36 materials tested, four were found to have unusual capacity to penetrate and kill Fusarium oxysporum f. cubense at depths down to 7 inches at dilutions of 200 ppm.

PROCEDURE

As dry, sifted, autoclaved fine sandy or silt loam at Changuinola, Panama, was poured into 1 x 10 inch vertical glass percolation tubes, agar discs of sporulating Fusarium oxysporum f. cubense, cut with a sterile cork borer, were placed at 7-, 5-, 3-, and 1-inch depths. Soil was then packed by dropping each tube, while vertical, 12 times on the table. Into a set of four such tubes, four dilutions of the candidate fungicide were poured until a few drops fell from the lower end. From 55 to 65 ml and a period of 10 minutes usually were required to saturate. Soil columns were supported on stainless steel wire screens resting on small glass protuberances inside the base of the tubes. After standing 24 hours, the wet soil was carefully removed with a home-made, sterile wooden spatula or paddle, and the discs thus recovered were plated out on Martin's agar plus streptomycin sulfate. Records on viability were taken 7 days later. Filter paper discs, held 2 weeks in broth shake culture of Fusarium, were as satisfactory as the agar discs and were easier to recover from the soil. With a little practice, 50 or 60 chemicals can easily be put under test in a day.

RESULTS

As evident from Figure 1, a 1% formalin solution killed at all four depths in the tube on the right, while the 1/2% solution failed to kill at the lowest depth, and the 1/4% solution merely slowed up growth from the two upper discs. The 1/8% solution had almost no effect at any depth in 24 hours' time.

Five fungicides of those tested killed Fusarium oxysporum cubense at dilutions 25 to 100 times as high as 1% formalin (Table 1).

Other fungicides tested, which failed to kill below 1 inch at these dilutions, are as follows: Arasan; Captan RE-4207 and RE-4297; Chemagro C-272; dichlorisopropyl ether; dichlorobutenes mixed isomers; Ethionine; Granosan and Granosan M; Guanyl 2129; Josite; Karathane; Omadine 4-0-1926, 6-1-1806, and 6-1-1902; Orthocide 50; ONCB; Parzate; Phaltan HL-877; Semesan; Solbar; Spencer 545 I, 1099 G, 1023 I, and 1184 G; Tersan; Trichloroethane; Tuzet; Verdosan; and zinc oxide treated (phenol). There was some retarding effect at the one-inch depth with 200 ppm concentrations exhibited by the Omadines, Chemagro C-272, and the mercurials. In one test with RE-4334 and 4371 in which an excess of chlamydospores of Fusarium was added to the agar, the results were the same. The reason stronger solutions were not employed, except of formaldehyde, is that by calculation 200 ppm active represents approximately 400 pounds per acre (2,000,000 pounds of soil). There is a question whether most fungicides could be economically employed at rates above 400 pounds, except such as cyanamide, which can be sold by the ton and has to be used in similar amounts.

For those more interested in the extent of detoxification exerted by different soils on the fungicide, it should be pointed out that the cup on the bottom of the percolation tubes, held on by rubber bands attached to glass hooks, is easily removed. Its contents can then be assayed by comparing its spore-killing capacity with that of the original solution to see what proportion has been lost. Such work is useful in determining dosage rates for muckland versus clay versus sandy soils.

If penetration studies are to be made deeper than a foot, it is desirable to use a series or stack of cylinders with close fitting joints. Both plastic and aluminum tubes have been used,

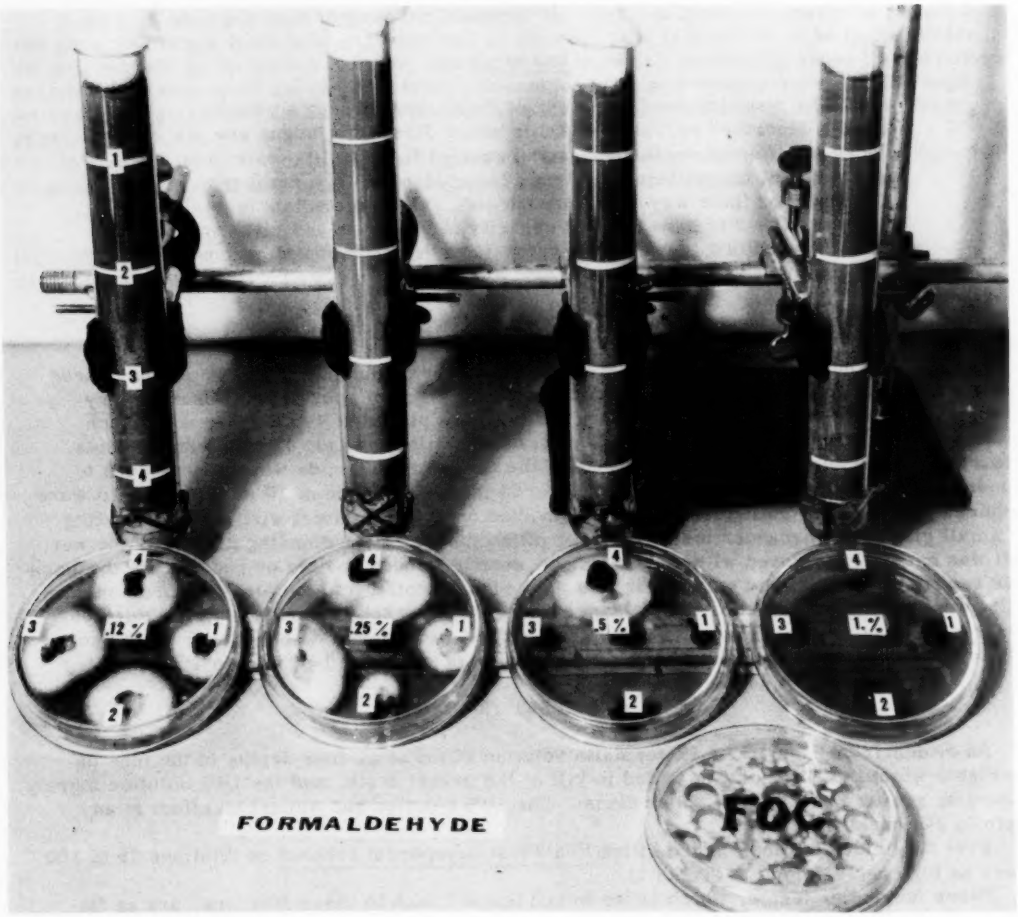

FIGURE 1. Equipment used and typical results obtained with one effective fungicide.

Table 1. Survival of Fusarium oxysporum cubense after 24 hours in soil drenched with different concentrations of selected fungicides.

Material tested	Inches deep	ppm 200	100	50	25	12
Cal. Spray RE-4371	1	-*	-	-	-	-
	3	-	-	-	-	-
	5	-	-	-	+	-
	7	-	-	+	++	-
Cal. Spray RE-4334	1	-	-	-	-	+
	3	-	-	-	+	+
	5	-	-	+	++	+
	7	-	-	++	++	++
Diamond Alk. 6-N-49	1	-	-	-	-	+
	3	-	-	-	+	+
	5	-	-	++	++	++
	7	-	+	++	++	++
Mylone 85-W	1	-	-	+	++	
	3	-	+	+	++	
	5	-	+	++	++	
	7	-	++	++	++	
Chemagro D-113	1	-	+	++	++	
	3	-	+	++	++	
	5	+	++	++	++	
	7	++	++	++	++	
Formalin**		1%	.5%	.25%	.12%	
	1	-	-	+	++	
	3	-	-	+	++	
	5	-	-	++	++	
	7	-	++	++	++	

* - = dead, + = retarded, ++ = no effect.

** 1% = 10,000 ppm so first three materials are 100 x as effective as formalin, or 37 to 40 x on basis of active formaldehyde.

the chief trouble being leaky joints and the time required to assemble and take down.

With glass equipment, Pote and Thomas (1) found that soluble fungicides could pass through 5 cm of soil without losing much lethal capacity. Zentmyer (3, 4) found longer periods of exposure than 24 hours had little or no effect. Furthermore, insoluble materials which performed poorly as drenches might be rather effective when thoroughly mixed with soil before water was added. All agree a test in soil is more reliable than a spore germination test on a slide or a test on agar in a petri plate.

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DEPARTMENT OF TROPICAL RESEARCH, UNITED FRUIT COMPANY (work done while on leave from CORNELL UNIVERSITY)

CYCLOHEXIMIDE (ACTI-DIONE[1]) AND ITS AGRICULTURAL USES[2]

Jared H. Ford, William Klomparens and Charles L. Hamner[3]

The presence of an antifungal antibiotic in culture filtrates from streptomycin-producing strains of Streptomyces griseus was reported in 1946 by Whiffen and co-workers (128). It was soon isolated in crystalline form by Leach and co-workers (70). The preliminary analyses and molecular weight determinations suggested a molecular formula of $C_{27}H_{42}O_7$ for the antibiotic and $C_{27}H_{44}N_4O_7$ for a dioxime. On the basis of this information the antibiotic was named Acti-dione: acti- from the fact that the organism which produced it was an actinomycete and -dione because the compound was thought to be a diketone. However, further studies by Ford and Leach (33) indicated that the molecular formular was $C_{15}H_{23}NO_4$ and that the compound contained only one carbonyl group. The chemical structure was determined by Kornfeld, Jones, and Parke (69) to be β - [2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] -glutarimide (Figure 1). The generic name, cycloheximide, was derived from its chemical name.

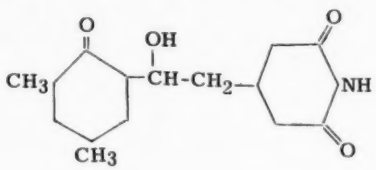

FIGURE 1

Brown and Hazen (11) have isolated cycloheximide from Streptomyces noursei and Morimoto, Oku and Nakamura (87) have obtained it from an unidentified Streptomycete.

In September, 1947, a sample of crystalline cycloheximide was submitted to the Chemical-Biological Coordination Center of the National Research Council for its screening program. Within a few months Irma M. Felber and Charles L. Hamner of Michigan State University who were participating in this program turned up a very interesting lead. In studying new compounds for possible growth effects they applied cycloheximide to bean plants. The antibiotic proved to be toxic to the young plants at concentrations above 100 ppm but 1- to 5-ppm solutions were found to control the powdery mildew (Erysiphe polygoni) with which they were infected. This was the first reported observation of plant disease control by an antifungal antibiotic (30) and was given wide publicity by a prominent weekly magazine in an article (114) which contained the speculation that Acti-dione might save the lives of the farmers' plants as penicillin and streptomycin had saved the lives of people. Within a short time requests for samples were received from many excellent investigators, but it soon became apparent that Acti-dione would not be "the penicillin of the plant world" as suggested by the magazine writer. Although it proved to be active against many fungi at concentrations of 1 to 10 ppm, it also exhibited phytotoxicity to certain species of plants at equally low concentrations. The problem became one of finding cases where there was a favorable ratio between the fungicidal activity and the phytotoxicity. Cycloheximide has been in commercial use for turf disease control since 1952 and for cherry leaf spot since 1954. The status of cycloheximide and other antibiotics in the control of plant diseases has been reviewed by Vaughn and Hamner (118), Anderson and Gottlieb (1), Leben and Keitt (71), Brian (10), Dunegan (28), Zaumeyer (133), and Lees (72).

IN VITRO ACTIVITY

Cycloheximide has been found to be remarkably specific in its action on microorganisms. Whiffen (125) reported that it inhibited the growth of certain yeasts at concentrations of about 0.1 and 1.0 ppm, while 1000 ppm failed to inhibit certain other yeasts or any of the 12 species

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1 Registered trademark.

2 A second paper entitled "Cycloheximide and its Non-Agricultural Uses" will be submitted to Antibiotics and Chemotherapy.

3 Department of Fermentation Research and Development, The Upjohn Company; Department of Agricultural Research and Development, The Upjohn Company; Department of Horticulture, Michigan State University, East Lansing, Mich., respectively.

of bacteria which were tested. These results have been confirmed and extended by others. A study of 33 species and strains of phytopathogenic fungi by the same author (126) revealed that they were all completely inhibited by 100 ppm. A few (Ustilago tritici, Diplodia zeae, Cladosporium fulvum, Sphaeropsis elysii, and Rhizoctonia sp.) were completely inhibited for 72 hours by about 1 ppm or less.

Wallen, Sutton, and Skolko (122) studied seven species of soil-borne phytopathogenic fungi and reported that the spores of Aschochyta pisi and Colletotrichum lindemuthianum were completely inhibited by 1 ppm. However, the growth of mycelial colonies of these same fungi was not inhibited by 225 ppm of the anitibiotic. The oak wilt fungus, Ceratocystis fagacearum, has also been found (Fergus and co-workers [32]) to be strongly inhibited by 0.1 and 1.0 ppm of cycloheximide.

Certain free-living protozoa are very sensitive to cycloheximide. Loefer and Matney (77) have reported that various strains of Tetrahymena gelii and Euglena gracilis are inhibited by 1 ppm or less. In screening for potential algicides, Palmer and Maloney (93) observed that cycloheximide was selective in its action. A 2-ppm solution proved to be toxic to Scenedesmus, Gomphonema and Nitzschia but not to Cylindrospermum, Microcystis or Chlorella. Laboratory tests on the rice stem borer, Chilo simplex, indicated that cycloheximide was the most toxic of the eight antibiotics that were investigated (112). Median lethal concentration by the wick method was found to be about 50 ppm for first instar larvae and 2500 ppm for fourth instar larvae. By intraperitoneal injection the median lethal dose was about 1.2 mcg. per adult insect.

Carter and Lockwood (14) reported that 125-1000 ppm of cylcoheximide lysed living mycelium of Glomerella cingulata growing in a liquid medium and on agar plates but did not lyse the dead mycelium.

USE IN SELECTIVE CULTURE MEDIA

The high degree of activity which cycloheximide exhibits against many yeasts, molds, and saprophytic fungi combined with its low activity against bacteria and certain pathogenic fungi has led to its use in several types of selective culture media.

Jeffers (61) reported that several phytopathogenic bacteria can be cultured on nutrient-dextrose agar containing up to 1000 ppm of cycloheximide. Holmes (58) suggested that cycloheximide might be of value in controlling mold contamination during the isolation of Graphium ulmi, the Dutch elm disease fungus. Schneider (103) reported that the addition of 200 ppm of cycloheximide and 10 ppm of streptomycin sulfate to potato-dextrose agar facilitated isolation of G. ulmi from elm specimens. The contaminants were greatly reduced and the antibiotics had little effect on the fungus.

In the isolation of actinomycetes from acid forest soils, Corke and Chase (19) found that the addition of cycloheximide to the agar plating media greatly inhibited the growth of the fungus contaminants. A 5-ppm concentration of the antibiotic greatly reduced the radial growth of the fungi, while 100 ppm did not inhibit any of the 85 actinomycete cultures that had been isolated on cycloheximide-free media.

CHERRY LEAF SPOT CONTROL

One of the most interesting and practical uses for cycloheximide is for the control of cherry leaf spot (Coccomyces hiemalis). In 1949 Petersen and Cation (94) applied 1- to 10-ppm solutions to heavily infected trees and 5 weeks later observed that the foliage on the cycloheximide-sprayed trees was greener and brighter and considerably more abundant than on trees sprayed with other fungicides (Figure 2).

Further studies by Cation (15, 16, 17, 83) and by other investigators (39, 46, 48, 82) during the past 5 years have shown that 0.5- to 2.0-ppm solutions give effective control, a remarkably low dosage as compared with older treatments (e.g., Bordeaux mixture, "insoluble" coppers, dithiocarbamates) which are used at 2000 to 8000 ppm. Furthermore, the older fungicides are generally prophylactics, while the cycloheximide will eradicate established infections (46, 83, 94).

Another important advantage of cycloheximide over the older fungicides is that its effect is not destroyed by heavy rains. Hamilton and Szkolnik (46), in artifical washing experiments with artificially infected potted cherry trees, demonstrated that a 2-ppm cycloheximide spray required about an hour on the leaves to give maximum effect. Any sprayed leaf with functional stomata was found to be protected, but there was no indication of translocation from one

FIGURE 2. Control of cherry leaf spot (Coccomyces hiemalis): Untreated on left; sprayed with cycloheximide (2 ppm) on right. (Courtesy D. Cation, Michigan State University)

leaf to another.

Although concentrations up to 10 ppm have been used after harvest with no apparent injury (94), the young, opening leaves were found to be more sensitive to the antibiotic and were sometimes twisted and stunted (15). Since the later leaves were normal, this injury was believed to be of little consequence. The injury was less apparent at 1 ppm than at 2 ppm and was more noticeable on sweet cherries than on sour cherries.

When cycloheximide was applied at petal fall, delayed ripening of the fruit was observed (15, 16, 48, 82), although this effect has not been consistent. In one year the fruit was dwarfed, but in another year it was larger with a reduced set (15). When applied to trees in full bloom the fruit set was reduced up to 100 percent, but little or no thinning was observed when the blossoms were closed (17). The reduction of fruit set may be advantageous on young trees and cycloheximide has been of particular value in nurseries where leaf spot was difficult to control (17). In order to minimize the possibility of undesirable effects on the fruit, it has been recommended, in some areas, that the first cycloheximide spray on bearing trees be delayed until the fruit has reached a diameter of about 3/8 inches (18).

Mackay (79) in Australia applied 1-, 5-, 10-, and 25-ppm solutions of cycloheximide to ripe cherries both by dipping in solutions and by spraying on the tree. No phytotoxic effects were observed and the fruit on the tree ripened normally. Taste panel studies indicated no effect on the flavor of the fruit. When 1-ppm or 2-ppm solutions were applied 14 days before harvest (111) and the resulting fruit was compared with unsprayed controls, a taste panel detected no difference in flavor, and there was no visible injury to the fruit. Taste panel studies at Michigan State University (2) also indicated that applications of 1 ppm 2 weeks after petal fall, 1 ppm 4 weeks after petal fall, and 2 ppm 1 week before harvest, did not change the flavor of the fruit, either at harvest or 6 months after it had been canned or frozen.

Residue studies (97) indicated that a 2-ppm spray on ripe fruit leaves a residue of about 0.02 ppm immediately after the spray has dried. In cool weather the half-life period of cycloheximide was found to be about 1 day. The fruit apparently contains an enzyme system that is capable of inactiviating the antibiotic.

Some fruit stem injury observed when cycloheximide was used in combination with lead arsenate was found to be due to omission of the usual addition of lime to the lead arsenate because of its adverse effect on the stability of cycloheximide in a tank mixture. Other lead arsenate correctives (ferbam at 1/2 to 3/4 pound per 100 gallons, iron sulfate at 1/2 pound per 100 gallons, or chelated iron at one pound per 100 gallons) may be used with satisfactory results. Other insecticides (parathion, malathion, dieldrin, DDT, methoxychlor, lindane, and nicotine) appear to be safe for use with cycloheximide on cherries. A few cases of chlorosis and premature leaf fall have been observed when cycloheximide was applied after dithiocarbamate sprays. Attempts to duplicate this damage under controlled conditions have been unsuccessful (18).

Cycloheximide was used commercially for post-harvest applications and on non-bearing trees in 1954. It was approved for pre-harvest use (within four days of harvest) in 1955 by the United States Department of Agriculture and the Food and Drug Administration.

TURF DISEASES

The use of cycloheximide on turf was initiated in 1949 by Hamner and Vaughn of Michigan State University. A 300-ppm solution controlled a new disease of creeping bent grass which was subsequently called melting-out and found to be caused by Helminthosporium sativum (67) (Figure 3). In tests in 1950 61 percent control of large brown patch (Rhizoctonia solani) was obtained in New Jersey (23), and almost perfect control of dollar spot (Sclerotinia homeocarpa) and melting-out in Michigan (117). No phytotoxicity was noted in either test. Control of dollar spot and melting-out has been confirmed by other workers (59, 68, 85) using a spray comprised of 0.6 g of cycloheximide and 14.0 g of ferrous sulfate per 1000 square feet of turf area.

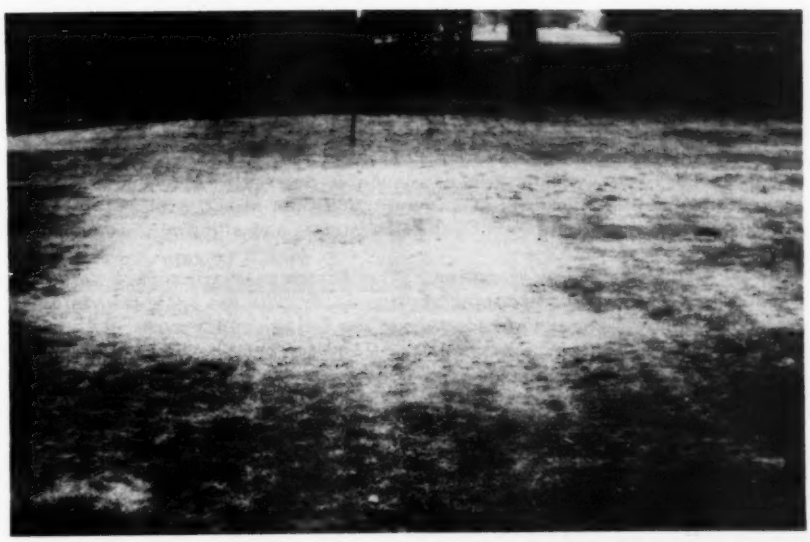

FIGURE 3. Melting-out disease (Helminthosporium sativum) on a golf course green composed of creeping bent grass.

Following the recognition of another prevalent turf disease, fading-out (Curvularia spp.), Howard (59, 60) reported control by cycloheximide.

Preliminary indications of control of going-out (Helminthosporium vagans) on Kentucky bluegrass and its relatives were reported by Howard (59). Inhibition of spore germination was obtained in a solution containing a few ppm of cycloheximide. In field tests, Couch and Cole (21) obtained good control of Helminthosporium vagans on Kentucky bluegrass from the application of 60-ppm sprays at 10- to 14-day intervals during the cool wet spraying period (April to May).

Snow mold (Fusarium nivale and Typhula spp.) of turf was well controlled in Michigan in 1950, 1951, and 1952 by 0.8 g of cycloheximide per 1000 square feet (applied in 2 gallons of water) when the ground remained frozen and snow cover was more or less constant (89). In eastern Washington, however, applications at unstated rates were ineffective (84).

Wells and Robinson (124) observed that cycloheximide at 0.6 g per 1000 square feet (applied in 10 gallons) gave control of cottony blight (Pythium aphanidermatum) on rye grass; however, under the 1953 Georgia conditions, daily applications were required.

The susceptibility of Merion blue grass to rust (Puccinia graminis) has created new problems for the turf specialist. Shurtleff (104) obtained promising results with cycloheximide used at 5.0 ppm with 45 ppm of ferrous sulfate 48 hours before and 48 hours after inoculation of seedlings in the greenhouse. The reduction of pustule formation was 97 to 100 percent from the protective treatment and 79 percent from the eradicative treatment. In 1956, Couch and Cole (20) reported that cycloheximide gave satisfactory control when applied as a 55-ppm solution at the rate of 2 gallons per 1000 square feet.

The beneficial effect of ferrous sulfate used in combination with cycloheximide was first noted by one of us (W. K.) in 1951. This effect was observed both in the laboratory (agar plate cultures) and in field tests. These observations were confirmed by Howard (59) in 1953. A commercial use of the antibiotic for turf disease control employs this combination. The mechanism of action is not understood since the ferrous sulfate has little or no antifungal activity when used alone at the same concentration.

OTHER PLANT DISEASES

Alfalfa: In an agar plate assessment cycloheximide completely inhibited Pythium debaryanum and P. ultimum at concentrations of 14.5 and 23 ppm respectively. Damping-off of alfalfa was prevented when the soil solution contained 6.2 to 25 ppm of the antibiotic, but these concentrations severely inhibited growth of the alfalfa seedlings (43).

Apple: Using 1- and 2-ppm sprays, Beraha, Wilson, and Dunegan (5) reported fair to poor control of powdery mildew (Podosphaera leucotricha), with extensive leaf injury on Rome Beauty trees. Hamilton and Szknolnik (47) found that 1 ppm mixed with wettable sulfur (2 pounds per 100 gallons) was effective but gave leaf mottle and stunting of leaves on sensitive varieties.

Swartout (110) found that cedar-apple rust (Gymnosporangium juniperi-virginianae) lesions were arrested by two applications of 1 ppm applied as soon as the rust was visible. However, an undesirable though not severe black spotting of the foliage and a moderate amount of black spotting on the under sides of the fruit resulted. Hamilton and co-workers (49) observed that cycloheximide oxime at 40 ppm inhibited further development of the cedar-apple rust fungus on the foliage of potted Rome Beauty trees when applied 24 hours after inoculation or when the lesions were just becoming visible. (For further discussion of cycloheximide oxime see "Systemic Activity of Cycloheximide Derivatives" below).

Azalea: Gill (38) reported that petal blight (Ovulinia azaleae) was not controlled by 5-ppm sprays).

Bean: Although the first observations of Felber and Hamner (30) on the protective action of cycloheximide were made on bean plants infected with powdery mildew (Erysiphe polygoni), further studies by Lockwood and Vaughn (76) indicated a very narrow margin between dosage levels which gave control and those which injured the plants. Effective control was obtained at 2 to 4 ppm, but when several varieties were sprayed with 10-ppm solutions some showed no injury whatever while others demonstrated various necroses, curling, and other abnormalities. The leaves of still other varieties were completely killed.

In greenhouse tests, Kenaga and Kiesling (62) obtained a moderate degree of control of Colletotrichum lindemuthianum (cause of anthracnose) on Michelite beans by application of a 3-ppm cycloheximide spray. Davison and Vaughn (24) reported that cycloheximide at 5, 50, and 100 ppm had little effect on bean root rot caused by Fusarium sp. in laboratory and green-

house trials.

Severe phytotoxicity was evident at the higher concentrations.

Blueberry: Zuckerman (135) observed that three 100 ppm cycloheximide sprays failed to control crown gall (Agrobacterium tumefaciens) on highbush blueberry bushes. Severe defoliation was observed with this treatment.

Broccoli: Natti (90) reported that cycloheximide in concentrations as low as 1 ppm inhibited the germination of downy mildew (Peronospora parasitica) spores in vitro. However, it did not control the disease when used in the greenhouse at 10 ppm on artificially inoculated broccoli plants.

Carnation: In studying the effect of fungicides on the mist propagation of carnation cuttings, Skiver (106) found that 2 ppm of cycloheximide, although effective against Alternaria dianthi on potato-dextrose agar plates, was unsatisfactory in practice because of its phytotoxic effect on the cuttings.

Cabbage and Chinese Cabbage: Kenaga and Kiesling (62) obtained a high degree of control of black-leg (Phoma lingam) in greenhouse tests by a 3-ppm application of cycloheximide.

Cedar: Strong and Klomparens (109) have reported that a single 100-ppm application prevented germination of teliospores and production of sporidia of Gymnosporangium juniperi-virginianae and G. globosum. The solution was applied to the galls on red cedar in the spring. No foliage injury was observed. By using 0.5% of summer oil in the spray mixture, effective control was obtained at 25 ppm and 50 ppm.

Cherry: Leaf spot control, which has been outstanding, is discussed in a separate section above.

McClure and Cation (83) observed that 2-ppm sprays applied for leaf spot also controlled powdery mildew (Podosphaera leucotricha) where it was also present. Swartwout (111) also reported good control of this mildew with 1- and 2-ppm solutions. Gilmer (39) obtained outstanding control of P. oxyacanthae on nursery stocks with 2 ppm in combination with sulfur (2 to 5 pounds per 100 gallons). Cycloheximide alone at 2 ppm was not highly satisfactory, although it gave control comparable with that obtained from copper fungicides.

Corn: In greenhouse tests, Kenaga and Kiesling (62) obtained good control of rust (Puccinia sorghi) on Golden Cross Bantam corn with a 3-ppm application of cycloheximide. Williams and Lockwood (129) found that at 50 ppm cycloheximide was toxic to sweet corn but gave some control of bacterial wilt (Bacterium stewartii) when applied before inoculation. This result was unexpected in view of the very low antibacterial activity of the antibiotic.

Cucurbits: deZeeuw and Vaughn (26) reported effective control of cucumber scab (Cladosporium cucumerinum) from 10-ppm sprays applied at 7- to 10-day intervals.

Ellis (29) applied 10-ppm sprays to summer squash and obtained good control of powdery mildew (Erysiphe cichoracearum), but this treatment was relatively ineffective against squash scab (C. cucumerinum).

Marlatt (80) observed that five applications of 1, 2, or 4 ppm caused no apparent injury to potted PMR45 cantaloup plants.

Dewberry: Young and Fulton (132) reported good control of powdery mildew (Sphaerotheca humuli) on Lucretia dewberry from one or two pre-harvest sprays of 2 ppm. No control was obtained from after-harvest sprays of 2 ppm or 5 ppm. No foliage injury was observed.

Elm: Tarjan and Howard (113) found that foliar sprays did not control Dutch elm disease (Ceratocystis ulmi) on young trees. A further indication of poor performance against this disease may be deduced from the fact that Holmes (58) suggested the use of cycloheximide to prevent mold contamination during isolation of the organism.

Grape: In 1954 tests Swartwout (110) found that the Concord variety was reasonably tolerant of cycloheximide at 1 ppm but was damaged at higher concentrations. Downy mildew (Plasmopara viticola) on a very susceptible variety, Thomas, was completely checked by 1 ppm. There was a moderate spread of the disease on the unsprayed controls.

In laboratory experiments, Ogawa and Vergara (92) tested cycloheximide sprays at 0.5 and 2 ppm for protective, systemic, and eradicative action against powdery mildew (Uncinula necator) on young Vitis vinifera plants. The treated plants were inoculated with conidia every 2 days for 5 weeks. The treated parts were practically free from infection but the new growth and the controls were severely diseased. The 2-ppm spray was highly eradicative but caused slight injury to the infected areas.

Juniper: Caroselli (13) reported that WK-34 gave good control of Phomopsis blight when applied to 1- or 2-year-old seedlings at the rate of 1 ounce per 10 gallons. This spray solution contained 12 ppm of cycloheximide and 675 ppm of pentachloronitrobenzene.

Lily: Pythium root rot on Croft lilies was not controlled when cycloheximide was used as a 100-ppm 60-minute bulb soak or as the bulb soak plus a 10 ppm soil drench, according to Scheffer and Haney (102).

Mint: Nelson (91) reported satisfactory control of spearmint rust (Puccinia menthae) with a 2% cycloheximide dust. The antibiotic was suggested as a possible replacement for sulfur fungicides, which leave undesirable residues in the distilled oil.

Mushroom: In selecting fungicides for the control of Dactylium mildew (Dactylium dendroides), Stoller, West, and Bailey (107) observed that cycloheximide was relatively effective when sprayed on the beds at 200 ppm. Goodman (40) reported that a 2.6 ppm soil drench on established mildew spots afforded 10 days of protection.

Oak: See section on Systemic Activity of Cycloheximide Derivates, below.

Orchid: Schaffner (101) found that cycloheximide appeared to be the most promising of several antibiotics tested for inhibition of fungus contamination of seed grown on agar media. Cattleya seeds sown on agar containing as much as 100 ppm germinated and the seedlings developed as well as in controls. When seedlings were transplanted from normal to cycloheximide media, some toxicity (root damage) was observed at the higher dosage levels of 50 and 100 ppm.

Peach: Ineffective control of brown rot (Monilinia fructicola) by 2 to 20 ppm was reported by Petersen and Cation (94). No foliar damage was reported but the higher concentrations cracked firm-ripe Halehaven peaches. In post-harvest dip treatments, Heuberger and coworkers (56) obtained serious injury at 5 to 20 ppm. In laboratory experiments, Ogawa and Vergara (92) reported that 100 ppm gave excellent protection against brown rot. In eradicant tests, fruits dusted with conidia and sprayed with 50 ppm after incubating for 1 day remained normal, but all of the controls developed brown rot.

KenKnight (63) obtained no control of peach rosette virus by immersing the roots of seedlings in a 25-ppm solution.

Pine: Cycloheximide was found by Moss (88) to be very effective for treatment of blister rust (Cronartium ribicola) trunk cankers on western white pine trees. The bark was removed from the diseased areas to expose the mycelium and 150-600 ppm oil solutions of the antibiotic were applied generously to the cut surface of the wounds. The wood-rotting fungi which were in evidence when the dead bark was removed also appeared to have been killed by this treatment.

Potato: Hilborn (57) observed that infection of potato stems by Rhizoctonia solani and Verticillium albo-atrum was reduced considerably by cycloheximide applied in watering solutions.

Raspberry: Ineffective control of powdery mildew (Sphaerotheca humuli) on Latham variety by 5-ppm sprays, reported by Fulton (34), was believed to be due to inactivation of the cycloheximide by a lime residue in the spray tank. Beneke and Young (4) found that pre-harvest sprays reduced the mold count in canned or frozen black raspberries.

Rose: Hamner and Vaughn (50, 119) obtained excellent control of powdery mildew (Sphaerotheca pannosa var. rosae) on greenhouse-grown hybrid teas. At 2.5 ppm some injury was observed on the new foliage of certain varieties, manifested in distortion of the youngest leaves. At 5 to 10 ppm some of the leaves became chlorotic and exhibited small necrotic areas. Swartwout (110) observed injury on some varieties of greenhouse roses at concentrations as low as 0.1 or 0.2 ppm under certain conditions when cycloheximide was used alone. The injury was confined to the very young leaves and was evident within 2 days after the application. By using a spreader (Triton B-1956), the injury at 0.5 ppm was reduced to a very low level on the sensitive variety, Pink Bountiful. In field trials on Dorothy Perkins, two 0.5-ppm applications almost completely removed the growth of powdery mildew on the leaves and flower buds without appreciable injury. A cycloheximide solution applied when the bushes were put into storage did not control Botrytis sp. on stored roses (99).

Safflower: Gattani (37) reported that cycloheximide gave promise in in vitro tests for control of Pythium sp. but its phytotoxicity precluded practical use.

Strawberry: Good control of leaf blight (Dendrophoma obscurans) with 5- to 50-ppm solutions was reported by Fulton and Cation (36). No phytotoxicity was mentioned and tests for cycloheximide residues on the fruit at harvest were negative.

Fulton (35) reported that 250- and 500-ppm solutions did not inactivate Type 2 strawberry virus. Miller and Vaughan (86) found that foliar sprays of 1 and 10 ppm did not inactivate the yellows virus complex in infected Marshall strawberry plants. No phytotoxicity was observed.

Tobacco: Todd (115) found cycloheximide to be ineffective in the control of blue mold

(Peronospora tabacina).

Wheat: Employing a bioassay method and paper chromatography, Wallen and Millar (121) were able to demonstrate that Garnet wheat locally absorbed and retained cycloheximide applied as 50- to 500-ppm sprays. The same concentrations applied as a soil drench resulted in root absorption and translocation to the leaf tissues present at the time of the application. No movement to tissues developing after treatment was observed.

Preliminary studies on the control of leaf rust (Puccinia rubigo-vera) and stem rust (P. graminis tritici) with cycloheximide were reported by Livingston (75). At 50 ppm it appeared to have some value, both as an eradicant and in imparting immunity to the plants, but the range between fungicidal effectiveness and phytotoxicity was found to be rather narrow. Silverman and Hart (105) reported that a 25-ppm spray controlled stem rust on Marquis seedlings by reducing the number of pustules and by delaying sporulation. However, a severe dieback of the seedling leaf tips was observed. In greenhouse trials, Wallen (120) observed that cycloheximide was the most promising of several antibiotics tested for control of stem rust. In field experiments with 25 to 50 ppm, on Garnet spring wheat, sprays containing 50-ppm or higher rates applied just before heading reduced rust intensity. Applications after heading reduced the rust slightly at 100 and 200 ppm, and by 50 percent at 500 ppm. Phytotoxicity was observed at concentrations as low as 50 ppm but the plants appeared to recover, and the yields were higher in the sprayed plots than in the unsprayed controls. A single spray applied 10 days after emergence was ineffective. Brentzel (8) reported effective control of black stem rust, Race 15B, with 1000 ppm in a greenhouse experiment. Subsequent field plot trials by the same investigator (9) gave very encouraging results. Three applications at 30 ppm gave good control of stem rust with no reduction in seed germination. Cycloheximide was stated to be the most effective of five fungicides investigated.

Crozier and Szkolnik (22) reported that 2 to 3 ppm of cyclohexmide was inferior to Karathane (2-capryl-4,6-dinotrophenyl crotonate) 1 pound per 100 gallons and micronized sulfur (6 pounds per 100 gallons) for controlling powdery mildew (Erysiphe graminis tritici) on greenhouse-grown wheat. The 3-ppm spray was reported to cause leaf injury.

Hagborg (45) observed that the addition of 2 to 4 ppm of cycloheximide to the culture solution failed to protect greenhouse-grown seedlings against infection by Xanthomonas translucens. This was not unexpected in view of the fact that this antibiotic is known to have very little antibacterial activity. Root dwarfing and reduction of the dry weight of the plants were also observed.

Mattern and Livingston (81) found that two applications of cycloheximide at 9 grams per acre had no effect on the mixing and baking properties of the flour obtained from the harvested seed.

SYSTEMIC ACTIVITY OF CYCLOHEXIMIDE DERIVATIVES

While testing certain derivatives of cycloheximide for eradicative activity against cherry leaf spot (Coccomyces hiemalis), Hamilton and co-workers (49) discovered that the oxime (33) was systemically effective. Potted Montmorency cherry trees in a greenhouse were sprayed with the oxime at 10 and 60 ppm, and with cycloheximide at 1 ppm. The trees were kept in the greenhouse 2 weeks for development of new growth, after which they were inoculated with C. hiemalis spores and held in a saturated humidity chamber for 2 days. On the new leaves, 42 leaf spot lesions per square inch developed on the cycloheximide-sprayed trees, as compared with only 10 lesions per square inch at 10 ppm and none at 60 ppm on the oxime-sprayed trees. Apparently the oxime was absorbed by the sprayed foliage and translocated to the new growth in amounts sufficient to afford protection. In further investigation of this significant discovery they found that other cycloheximide derivatives, i.e., the acetate, isomer, and semicarbazone, were also active systemically at 10 ppm or less, while the anhydro derivative was somewhat less active and the dehydro derivative was about as active as the oxime. When applied 4 days after inoculation, the acetate, isomer, and semicarbazone were protective at 0.5 ppm and gave good eradicative control at 1 ppm. These authors suggest that systemic control may make the timing of the fungicidal sprays less critical and reduce the number of applications required for protection.

In a radioactive tracer study, Lemin and Magee (73) found that cycloheximide acetate-2-C^{14} was absorbed by tomato roots and induced antifungal properties in the leaves. The antifungal activity was believed to be due to the formation of free cycloheximide in the foliar parts.

Phelps and co-workers (95) reported that cycloheximide and its acetate delayed the symptom expression of oak wilt (Ceratocystis fagacearum) in over one-half of the trees observed

for at least 3 months. Solutions of the two materials were applied by the chisel-cone method. Some phytotoxicity was noted.

The semicarbazone and acetate derivatives of cycloheximide induced pre-infection resistance to black stem rust (Puccinia graminis tritici) on Onas Wheat according to Hacker and Vaughn (44). The compounds were applied as 100- and 200-ppm sprays. Yields were increased but not significantly. Phytotoxicity was slight and there was no apparent effect on germination.

SOIL AND SEED TREATMENT

Vaughn and co-workers (119) reported that germination of cantaloup, spinach, and cucumber seeds was not inhibited by soaking in cycloheximide solutions up to 100 ppm for 30 minutes, but some other seeds were found to be much more susceptible to injury by the antibiotic. In 4-hour soak treatments, the approximate concentrations reported to give 50 percent inhibition of germination were: alfalfa, 100 ppm (43); pea, 100 to 225 ppm (119, 122); radish, 25 to 50 ppm (119); wheat, 10 to 20 ppm (119). In soil applications, 5- to 10-ppm solutions gave 50 percent inhibition of germination of red kidney beans (119). Henry and co-workers (52, 53, 54) reported that there was no appreciable injury to oats or barley by 10-ppm solutions but that wheat was injured. However, these authors found that 0.5% or 1% cycloheximide dusts could be used at 0.5 to 1.0 ounces per bushel on wheat without significant reduction of emergence except with the highest dosage. deZeeuw (25) found cycloheximide to be rather toxic to beets. Dosdall (27) reported that a 10-hour soak at 5 ppm killed calla rhizomes. Gattani (37) obtained the following results from germination tests with safflower seeds, using a 16-hour soak in the respective cycloheximide solutions: at 5 ppm, germination was 98 percent of control; 50 ppm, 55 percent; 500 ppm, 43 percent; 5000 ppm, 15 percent. Randhawa and Hamner (98) observed that the inhibitory action of cycloheximide on seed germination was greatly increased in the presence of a mixture of nine other antibiotics and was decreased in the presence of a mixture of three growth regulators.

The effect of soil on the microbiological activity of cycloheximide has been studied in detail by Gottlieb, Siminoff, and Martin (41). After application to soil at 5 to 20 ppm, water extraction removed 22 to 24 percent of the antibiotic at 24° C and about 90 percent at 80°. The microbiological activity of cycloheximide was found by Zentmyer (134) to be about the same in soil as in agar, but it varied somewhat in different types of soils. Rushdi and Jeffers (100) observed that addition of 5 percent hay to the soil reduced the activity of several fungicides, including cycloheximide. Cycloheximide was reported (100) to be more active in soil at pH 4.5 than at pH 6.1 or 7.1 but this may be due to lower stability in the alkaline soil, to be expected in view of its poor stability in alkaline water. Stability was greater in autoclaved than in freshly dug normal soil (41), indicating that soil microorganisms markedly hastened the inactivation of the antibiotic.

Streptomyces griseus can produce cycloheximide in sterile soil but only when suitable nutrients (i.e., soybean meal) are added (41).

Although cycloheximide has been reported to be active against several economically important seed or soil-borne organisms -- e.g., Ascochyta pisi (122), Fusarium poae (122), Colletotrichum lindemuthianum (122), Alternaria brassicicola (122), Ustilago kolleri (54), Tilletia spp. (52), Pythium spp. (42), P. debaryanum (1) -- no important uses for cycloheximide as a seed protectant have been developed up to the present time. Henry and co-workers reported encouraging preliminary results on barley, oat, and wheat smuts (52, 53, 54) but subsequent cooperative field tests in comparison with other fungicides gave only fair results (78). Gregory and co-workers (43) obtained significant protection of alfalfa against damping-off but the margin between the protective and phytotoxic dosages was very narrow. Seed protection studies on beet and spinach (25), sorghum (74, 96), pea (122), and red pine (108) have given only fair to poor results.

Harvey (55) has studied the effect of cycloheximide and three other antibiotics on soil microbial populations, plant growth and the nodulation of sweet clover.

WOOD PRESERVATION

Klomparens (65, 66) found that the wood rotting fungi Poria microspora, Lentinus lepideus, Fomes igniaruis var. laevigatus, Lenzites trabea, and Schizophyllum commune were completely inhibited for 48 hours when grown on a malt extract agar medium containing 2.0 ppm cycloheximide.

Under the same conditions, the following wood-staining fungi were completely inhibited by 0.1 to 10 ppm: Ascocybe grovesii, Endoconidiophora moniliformis, Hormiscium gelatinosum, and Ophiostoma adiposa, while Cadophora brunnescens and Curvularia geniculata required 100 ppm for complete inhibition. Seventeen other wood-staining fungi were found to be relatively insensitive to 100 ppm (31).

Fergus (31) reported that there was no increase in resistance to cycloheximide when A. grovesii was transferred from media containing 0.01 ppm to media with higher concentrations of the antibiotic.

In a preliminary test, Klomparens (65, 66) observed that cycloheximide appeared to be selectively adsorbed by basswood blocks dipped for 3 minutes in a 200-ppm solution. Subsequent assay of the solution indicated that only 100 ppm of cycloheximide remained. Using a modified Leutritz technique, a hot and cold bath treatment in 100-ppm cycloheximide solution appeared promising for wood preservation, being superior to cold soak and hot dip treatments.

OTHER STUDIES

Rodent Repellent Studies: Using food-acceptance as a screening method, cycloheximide was found to be by far the most effective of about 4,000 chemicals which were tested on laboratory rats (123). The rats refused to drink water containing as little as 1 ppm even when no other water was present (116, 123). In barrier tests measuring the time required for wild rats to penetrate test boxes coated with the repellent compounds, cycloheximide was found to be 20 times as effective on a weight basis as the next best compound (123). A species variation in repellent action was noted. Four species of mice were found to be more tolerant of the antibiotic than were laboratory rats (123). Of a series of 40 imides prepared and screened by the food-acceptance method some showed repellent activity but none approached cycloheximide in repellency (3). In chronic toxicity tests it was observed that white mice would drink apple juice containing 1 ppm but would refuse it when a 10-ppm concentration was present (64).

Cytological and Genetic Effects: The cytological effects of cycloheximide on onion and pea root tips were studied in detail by Wilson and co-workers (7, 51, 130, 131). Cycloheximide markedly lowered the mitotic index and also prevented completion of prophase in nuclei already in division at the time of treatment. Although cycloheximide caused "reductional groupings", it was concluded that the possibility of polyploidy or other permanent changes resulting was rather doubtful, since most of the affected cells appeared to be incapable of further divisions. Whiffen (127) reported that cycloheximide induced the formation of male and female gametangia by the sporophyte of Allomyces arbuscula. She suggested that the action might have been mitotic interference (vegetative reduction division) due to intensification of an existing condition. In studies with cedar-hawthorn rust (Gymnosporangium clavipes) Berliner (6) observed that cycloheximide at 5 to 500 ppm checked basidiospore formation, induced forking of basidia, and interfered with spindle function, which in turn affected synchronization between nuclear divisions and basidial cell wall formation. The cytological effect of cycloheximide on the fungus Blastocladiella emersonii was studied by Cantino and Hyatt (12) who found that cycloheximide treatment changed the ratio between two phenotypes and effected emergence of a third.

TOXICITY OF CYCLOHEXIMIDE

Crystalline cycloheximide and its concentrated solutions are potent skin irritants and should be handled with caution. The irritation is not apparent until about 6 to 24 hours after exposure, and consists of reddening and sloughing of the skin. Prompt washing of exposed skin areas with soap and water has been found to prevent the irritations. However, sterile 1% aqueous solutions of cycloheximide have been administered intramuscularly or intravenously to many patients with no reports of local irritation. In this respect cycloheximide resembles phenol. Phenol is also a potent skin irritant in crystalline form or in concentrated solutions but it is widely used at 0.5% concentration as a preservative in sterile solutions of drugs for intramuscular or intravenous administration. We are not aware of any reports of skin irritation from the use of solutions in the concentrations that are ordinarily employed for plant disease control (0.5 to 250 ppm).

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INHIBITION OF GROWTH OF CERATOCYSTIS ULMI IN VITRO BY RESIDUES FROM EXTRACTS OF SOILS AND OF PLANTS GROWING IN SOILS TREATED WITH CAPTAN OR ORTHOCIDE 50W

Curtis May, John G. Palmer, and Edward Hacskaylo[1]

Summary

A preliminary study on the possible absorption of captan from soil by several species of plants is reported. Ceratocystis ulmi, the organism causing Dutch elm disease, was found to be very sensitive to captan in vitro. Residues of acetone extracts from leaves or stems were tested by bio-assay on agar plates seeded with C. ulmi. Greater inhibition of the fungus in vitro regularly occurred around residues from several species of plants growing in soil treated with captan when the rate was between 1 pound and 10 pounds per 25 square feet than when the rate was 0.1 pound or when the soil was not treated. Captan was not identified in any extract, but circumstantial evidence indicates that it or a closely allied chemical was present in the extracts from plants growing in treated soil. Inhibitory residues were obtained from humus-containing soils in the greenhouse and in the field several months after application of captan.

INTRODUCTION

During the search for systemic fungicides that might control Dutch elm disease, an edgeburn of leaves present on inoculated and uninoculated elm trees growing in soil treated with captan or Orthocide 50W but absent on those growing in untreated soil suggested chemical injury. Studies were then made to determine whether a fungitoxin was present in the treated elms. Herein recorded are the methods used and the data obtained in experiments 1) to extract plant organs and soil, 2) to assay in vitro any fungitoxic property of residues from extracts by using Ceratocystis ulmi as the test fungus, and 3) to survey the responses of available shade tree and ornamental plants similarly treated with captan.

METHODS AND RESULTS

Elm

Three-year-old seedlings of American elm were planted in sterilized composted soil in 8-inch pots and set on a bench in the greenhouse. In February 1957, technical captan (94.7%) equal to 1 percent of the weight of the air-dry soil was distributed over the surface of the soil in each pot and scratched lightly into it. Soil in 30 pots was treated at the 1-percent rate twice and in 18 pots five times, at weekly intervals. Similar potted trees set on the same greenhouse bench served as untreated controls.

On May 1, 1957, 24 of the trees treated twice and all those treated five times were inoculated by cutting into the wood of each stem on opposite sides and then placing two drops of a heavy spore suspension of C. ulmi in each cut. The cuts were then wrapped firmly with rubber bands. Twelve trees not treated with captan were similarly inoculated. Data on wilt and discoloration in the wood were recorded on May 28. All inoculated trees had vascular discoloration in both old and new wood. The record of the extent of wilt is given in Table 1.

After the addition of captan to the soil in which the plants were growing, both old and new leaves became stiff and brittle and some developed mild chlorosis before inoculation. Subsequently the tips and margins of old leaves which were present on both inoculated and uninoculated trees at the first treatment turned brown. This symptom syndrome suggested chemical injury and was associated with the captan applications. Experiments were conducted to determine whether a fungitoxic material could be extracted from plant organs and assayed in

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Table 1. External symptoms of Dutch elm disease in potted 3-year-old American elms growing in untreated and captan-treated soil and inoculated 4 weeks previously.

Number of applications	Inoculation	Trees in test	Trees with wilt symptoms of indicated type			
			None	Very mild	Mild	Severe
		Number	Percent	Percent	Percent	Percent
None	No	12	100	0	0	0
None	Yes	12	50	0	25	25
2	No	6	100	0	0	0
2	Yes	24	63	5	20	12
5	Yes	18	22	3	39	22

vitro against C. ulmi.

Captan is only very slightly soluble in water but is more soluble in acetone. Acetone extracts of 10-gm samples of leaves from the treated and untreated but uninoculated trees were made as described previously[2].

Following a 24-hour extraction the acetone was evaporated at 45°C, and the residues were tested by bio-assay for the presence of a fraction inhibitory to spore germination and mycelial growth of C. ulmi. Spores of the fungus grown in a nutrient solution containing inorganic salts, peptone, glucose, and yeast extract, were added to liquid potato-dextrose agar at 45° to give a dilution of 15,000 to 18,000 spores per ml. Petri dishes each containing 15 to 20 ml of the agar were prepared. The residues were accumulated and placed on the agar in the dishes as soon as it solidified. When there was too little residue to be removed from the porcelain evaporating dish, the soluble fraction was dissolved in 2 ml of acetone. Then filter paper discs, such as used in assays of antibiotics, were dipped in the solution, dried and handled as residues. The cultures were incubated for 60 to 72 hours at 18°. The sensitivity of C. ulmi to captan was tested similarly. Inhibition zones 19 mm wide were produced around discs dipped in a 1:1000 acetone solution of captan. Less concentrated solutions produced narrower zones.

Residues obtained in June and subsequently at 2-week intervals (four extractions) through August 1, 1957, from fresh leaves of treated American elms inhibited growth of C. ulmi; those from leaves of untreated elm trees did not.

By August 15 all trees were dormant as indicated by hardening of stems and cessation of growth. Residues from leaves collected from treated and untreated trees on August 29 failed to inhibit growth of the fungus. One of the treated trees started to grow, and the residue from 8 gm of the new leaves collected on September 9 inhibited C. ulmi. Residues from 15 gm of old leaves from treated and untreated trees failed to inhibit it. Residues from 10-gm samples of wood from treated and untreated trees collected on September 19 failed to inhibit the fungus.

Residues from 10-gm air-dry samples of the treated soil collected on September 9 and all residues previously obtained from treated soil inhibited growth of the fungus whereas residues from untreated soil did not.

Thirty American elm seedlings approximately 3 feet in height were established in number 2 glass sand in 5-inch azalea pots. The trees were divided into two lots on February 13, 1958. Ten trees were set in an asphalt-coated pan containing nutrient solution. The other 20 trees were set in a similar pan containing nutrient solution to which a slurry of Orthocide 50W had been added in a sufficient quantity to saturate the solution and provide an excess of the chemical. The solution in both pans was maintained 1/2 inch deep throughout the experiment by carefully adding water at one end of the pan. Leaf samples were taken from both lots of plants after treatment for 14 and 21 days. Residues of extracts of leaves from the

[2] May, C., J. G. Palmer, and E. Hacskaylo. In vitro inhibition of Ceratocystis ulmi by acetone extracts from leaves or stem of some species of higher plants. Plant Dis. Reptr. 42: 399. Mar. 15, 1958.

treated plants did not inhibit growth of C. ulmi in vitro more than residues of leaves from untreated plants.

On March 12 slurry of Orthocide 50W was prepared and was carefully added to the top of the sand in six pots of the lot standing in the nutrient solution-Orthocide 50W mixture. After 7 days leaf samples were taken from these six trees treated from both top and bottom of the pot. Leaf samples were also taken from the trees subirrigated with nutrient solution saturated with captan, and from the control trees that received only nutrient solution. Residues of leaf samples from individual trees receiving nutrient solution only produced zones of inhibition from 0.75 to 3.25 mm wide. Residues from each of the trees receiving Orthocide 50W by subirrigation only produced zones from 1.5 to 2.5 mm wide. Residues from those trees receiving Orthocide 50W by subirrigation and from the top of the pot produced inhibition zones from 3.7 to 9.5 mm wide.

Rose

On August 6, 1957, captan in the form of Orthocide 50W was distributed evenly on the surface of the soil around field-grown rose bushes and then was raked lightly into it. A sawdust mulch, which had been removed before the treatment, was replaced, and the area was then thoroughly wet by sprinkling. Of the 16 randomized 5 foot x 5 foot plots each containing two mature, unsprayed, unfertilized bushes of Red Radiance rose, four plots were treated at the rate of 0.1 pound, 1 pound, and 10 pounds per 25 square feet. Four raked and mulched plots to which no Orthocide 50W was added were maintained as controls.

Acetone extractions of rose leaves from each of the plots were made before the soil was treated. A naturally occurring fraction which uniformly inhibited growth of C. ulmi in bio-assay was present in each of the extracts as previously reported[3]. Residues made later from leaves of bushes growing in untreated plots also inhibited the fungus to a similar degree in the bio-assay tests.

Residues from rose leaves collected 24 hours after Orthocide 50W was applied were tested by bio-assay. The zones of inhibition were widest for residues from leaves from bushes grown in plots treated with 1 pound of Orthocide 50W. The zones were wider for the plots which received the 10-pound treatment than for the controls but for those which had received the 0.1 pound treatment, the zones were no wider than for the controls (Fig. 1). At 72-hour, 10-day, and subsequent samplings (at 2-week intervals through the middle of September), the zones of inhibition were widest for the 10-pound treatment. The zones for the controls and the 0.1-pound treatment were equal but narrower than those for the 1-pound treatment. Mature rose leaves and stems from which the leaves had been removed were collected 3 weeks after their initial treatment with Orthocide 50W at the rate of 10 pounds per 25 square feet. Residues from both the leaves and stems inhibited growth of C. ulmi more than residues from untreated controls. At no time and at no dosage was there any apparent injury to the rose plants. Residues from rose leaves randomly selected on September 26 as to age and location on bushes in all the treatment plots failed to inhibit growth of C. ulmi more than residues from leaves of untreated bushes. The width of the zones of inhibition ranged from 4 to 6 mm. Residues from leaves collected October 2 and separated into two groups designated as young leaves (not fully expanded and still reddish) and old leaves (fully expanded and dark green) caused no increased zones of inhibition as compared with the controls.

The characteristic odor of captan was still identifiable in the treated soil on October 2. All 10-gm samples (wet weight) of soil from the treated rose plots during the experiment and on October 2 yielded inhibitory residues; the relative diameters of the zones of inhibition were proportional to the concentration of Orthocide 50W applied to the soil. Residues from untreated soil were not inhibitory (Fig. 2). In a preliminary test, concluded after 70 days, residues from water extracts of a soil-captan mixture were inhibitory to growth of C. ulmi in the bio-assay test.

Pin Oak and Maples

Orthocide 50W was applied at the rate of 3 pounds per 16 square feet to soil surfaces around one each of 5-year-old pin oak, sugar maple, and Norway maple trees on September 6, 1957. Random, 15 gm samples of leaves were taken from each tree 24 hours, 72 hours,

[3] See footnote 2 above.

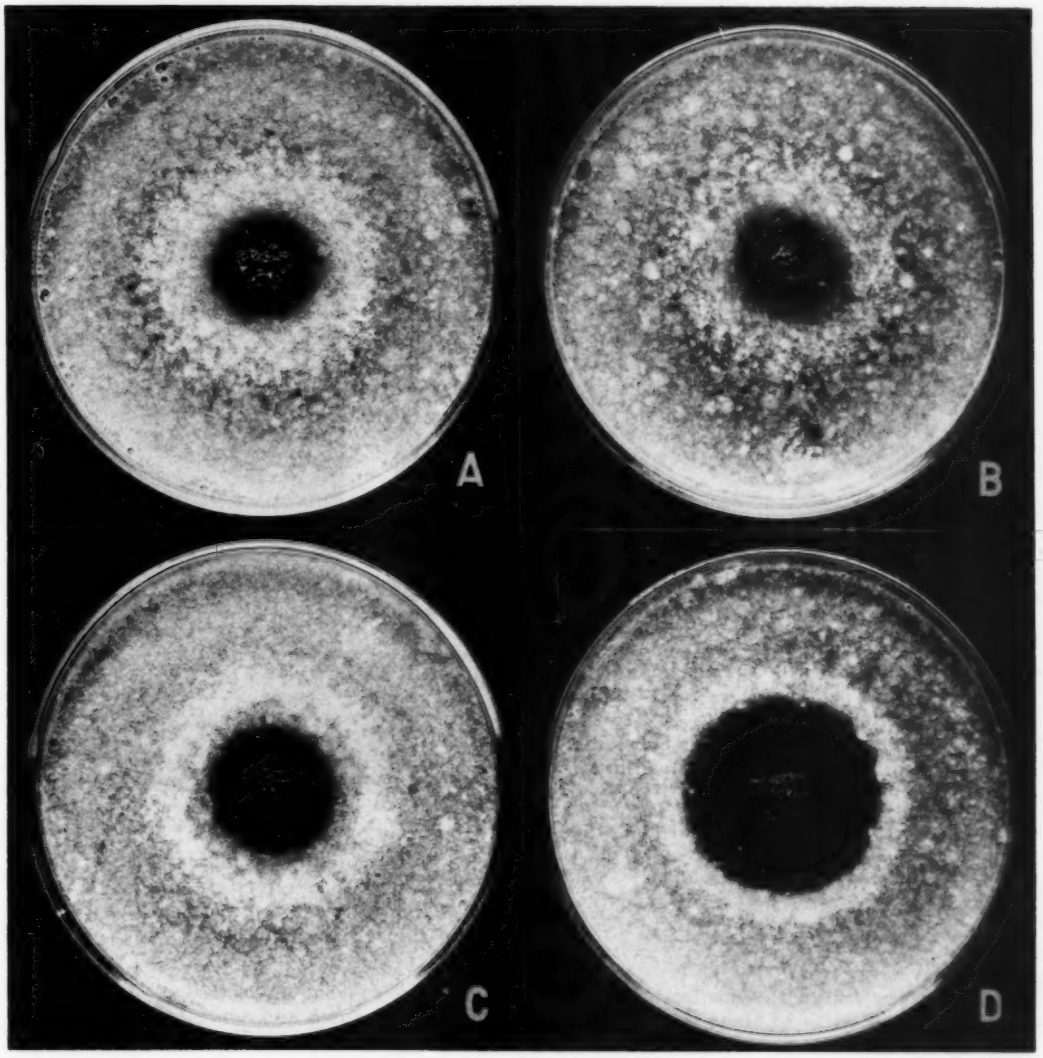

FIGURE 1. Growth of Ceratocystis ulmi as affected by residues from leaves of Red Radiance rose growing in untreated soil and in soil treated with Orthocide 50W: (A) untreated, (B) treated with 0.1 pound, (C) treated with 1 pound, and (D) treated with 10 pounds per 25 square feet. The average widths of the zones of inhibition were 3, 3, 5, and 15 mm, respectively.

10 days, and 3 weeks after treatment. In the bio-assay, residues from leaves of treated trees inhibited growth of C. ulmi much more than those from leaves of untreated 5-year-old trees of the same species. Among residues from leaf samples from the treated trees collected October 30, when autumn coloration was past its peak but before many leaves had abscised, the residues from the sugar maple alone inhibited C. ulmi. Residues from the others did not. Residues from the treated soil taken at the same time and at all previous times markedly inhibited C. ulmi as compared with those from untreated soil samples.

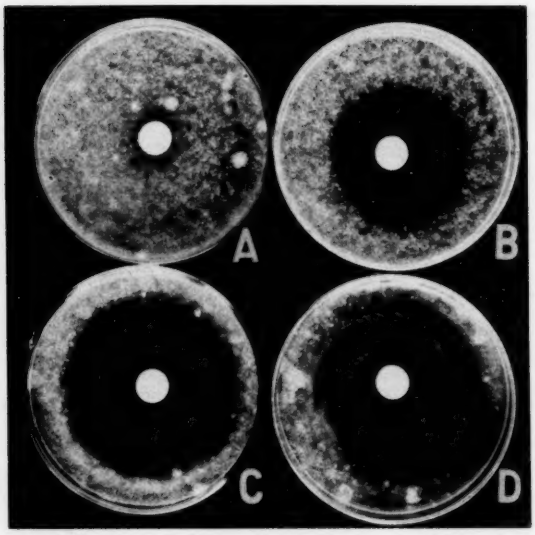

FIGURE 2. Growth of Ceratocystis ulmi as affected by residues from untreated soil and soil treated 59 days previously with Orthocide 50W: (A) untreated, (B) treated with 0.1 pound, (C) treated with 1 pound, and (D) treated with 10 pounds per 25 square feet. Widths of the zones of inhibition were 0, 15-21, 24, and 21-30 mm, respectively. Filter paper discs dipped once in residues redissolved in 2 ml of acetone were used in the assay.

FIGURE 3. Tip burn of wheat growing in soil treated with Orthocide 50W at the rate of 10 pounds per 25 square feet of soil surface. The lighter areas at the tips of the leaves are dry and light brown or tan.

Wheat

Sufficient Orthocide 50W was mixed with the soil in rectangular flats having inside measurements of 14 x 26 x 3 inches to give surface application rates of 10 pounds and 1 pound per 25 square feet. Little Club wheat was planted[4] in one-half of each flat on October 16, 1957, and in the other half on October 19. Similar untreated soil was planted with Little Club on the same dates. The heavier dosage retarded germination.

Residues of 15 gm of fresh leaves collected on October 29 from plants growing in treated soil produced zones of inhibition ranging in width from 5 to 8 mm. Those from leaves of control plants did not inhibit C. ulmi except in one plate in which there was a 1-mm fungus-free zone. Residues from leaves collected on November 4 from plants in soil treated at the 1-pound dosage rate and from the controls failed to inhibit growth of C. ulmi. However, the residues from the leaves of plants which received the 10-pound dosage were strongly inhibitory.

Tip burn developed on the leaves of treated wheat plants. It was most pronounced on those receiving the heavier treatment (Fig. 3), but was severe even on those given the lighter

[4] The generous cooperation of Wm. Loegering and Warren Giese, who supplied the seed and cared for the plants is acknowledged.

treatment. There was only very minor tip burn on leaves of control plants. When a 2.5-gm sample of the dry, dead tips and a 2.5-gm sample of air-dried portions of wheat leaves from treated plants were extracted, the residue from each inhibited growth of C. ulmi more than the residue from the air-dried living leaves of untreated plants. The residues from dead tips and those from the green parts of the same leaves of treated plants produced zones of inhibition 7 mm and 2 mm wide, respectively.

Tomato

Ten gm of Orthocide 50W was lightly scratched into the soil surface around six Rutgers tomato plants growing in the greenhouse in 4-inch pots (the rate of application approximating 5 pounds per 25 square feet[5]). In every instance the leaves of the treated plants soon appeared water-soaked and then developed severe injury. Residues from leaves of treated plants inhibited growth of C. ulmi more than those from leaves of untreated plants. The average width of the zone of inhibition caused by residues from treated plants was 16 mm and from control plants 10 mm. However, residues from the leaves of individual untreated tomato plants were extremely variable in their inhibitory effect. The narrowest inhibitory zone among the residues from an individual treated plant measured less than the widest zone among those from untreated controls.

Poinsettia

On December 2, five potted plants of Ruth Ecke poinsettia were treated by adding Orthocide 50W to the soil at the rate of 1 pound, and five plants were treated at the rate of 5 pounds, per 25 square feet. Residues from leaves removed after 24 hours from the plants treated at the 5-pound rate produced inhibition zones averaging 8 mm wide while those from the plants receiving the 1-pound rate and from the controls failed to inhibit growth of C. ulmi. Residues from leaves removed 1 week after treatment caused inhibition zones averaging 12 mm and 16 mm for the 1-pound and 5-pound rates, respectively.

Pine

Three replicates of 2-year-old, potted slash pine were treated by adding Orthocide 50W to the soil at the rates of 1 pound, 5 pounds, and 10 pounds per 25 square feet. Untreated controls were maintained. Residues from collections of needles made 1 week after treatment produced zones of inhibition averaging 2.5 mm for the 5-pound rate and 1.5 mm for the 10-pound rate. Residues from the pines treated at the 1-pound rate and from the controls did not inhibit growth of C. ulmi in bio-assay.

DISCUSSION

Chemicals that would prevent or restrict growth of C. ulmi within the host might be useful in control of Dutch elm disease, particularly if the chemical could be generally distributed in affected tissues including stems. In these experiments, if captan was absorbed by the roots of the elm trees, it concentrated in tissues of leaves rather than in stems, as indicated by the bio-assay test with C. ulmi.

Greater inhibition of C. ulmi in vitro regularly occurred around residues from organs (mainly leaves) of several species of plants growing in soil treated with Orthocide 50W when the rate of application was between 1 pound and 10 pounds per 25 square feet than occurred around residues from untreated control plants. At no time in these experiments was captan or a derivative identified as the agent responsible for inhibition zones about residues from treated plants. Consequently, it cannot be stated that captan was absorbed through the roots and concentrated in any tissues of the experimental plants. There is circumstantial evidence, however, that captan or a closely allied chemical was present in extracts from treated plants. The following findings support this view: 1) strong inhibition of C. ulmi by captan in bio-assay, 2) chemical injuries to leaves fully developed on elms at the time of the initial treatment with captan but absent from leaves on untreated plants, and 3) initially a greater inhibition by residues from plants treated at the rate of 1 pound per 25 square feet or more than from untreated

[5] The generous cooperation of S. P. Doolittle, who supplied the plants for the experiment, is gratefully acknowledged.

plants. Absence of increased inhibition around residues prepared in autumn from the developing leaves of the Red Radiance rose possibly may be attributable to decreased root absorption rather than to decreased movement into the leaves because of the formation of an abscission layer.

Inhibitory residues were obtained from humus-containing soil both in the greenhouse and in the field for several months after application of captan or Orthocide 50W. In bio-assay, residues from comparable untreated soils always failed to inhibit C. ulmi when compared with extracts from treated soils. The odor of captan was readily distinguishable after 2 months in the soil in the rose plots treated at the rate of 10 pounds of Orthocide 50W per 25 square feet and after 6 months in the greenhouse in pots treated with much greater concentrations.

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ACTI-DIONE STOVE OIL TREATMENT OF BLISTER RUST TRUNK CANKERS ON REPRODUCTION AND POLE WESTERN WHITE PINE

Virgil D. Moss[1]

Summary

Blister rust mycelia around wounds of partially excised trunk cankers on reproduction and pole western white pine were satisfactorily killed by 150 ppmw Acti-dione in No. 1 stove (fuel) oil. Trunk cankers were removed using a hatchet or knife by starting the cut 1 inch and 2 inches, reproduction and pole respectively, above the outer limit of surface discoloration and extending the cut downward along the visible canker margin to the proximal limit of surface discoloration. Treatment was completed by wetting cut surfaces to the dripping point with Acti-dione stove oil applied by fog dispersing sprayer. Number 1 stove oil proved particularly suitable as a carrier for Acti-dione. It is readily available at low cost and combines the following good features: high penetrating capacity, slow evaporation, satisfactory spreading properties, no objectionable odor or skin irritation, noncorrosiveness, less harmful to trees than comparable priced diesel oil, and noncongelating at winter temperatures. Two other carriers, Sovaspray 100 and aqueous 2% glycerol, proved unsatisfactory compared with No. 1 stove oil.

A damage-prevention measure to preserve adequate stocking of crop trees in stands of western white pine, Pinus monticola Dougl., that become infected by blister rust, Cronartium ribicola Fischer, before Ribes are destroyed was previously studied in the Coeur d' Alene National Forest, Idaho (3).

Trunk cankers were excised by cutting out bark from inside the margin of surface discoloration to expose blister rust and wood-rotting pathogens and bark beetle infestations, and remove necrotic tissue to facilitate callus formation. The antifungal antibiotic Acti-dione[2] (cycloheximide) at 150 ppmw in the isoparaffinic base oil Sovaspray 100 was applied to cut surfaces of trunk canker wounds with a small paint brush. This control measure killed 83 percent of the blister rust trunk cankers on pole-size western white pine. The results from this type of treatment were not improved by concentrations of 300 and 600 ppmw Acti-dione.

The survival of trunk cankers was attributed to the short distance Acti-dione penetrated phloem and cambium tissue from the edge of a cut surface. The distance of penetration was greatest in a downward and lateral course from the margin of a wound, and least in an upward direction against the outflow of elaborated foods. No evidence was found of surface bark penetration on pole-size western white pine.

Tests in 1956 to compare the penetration of aqueous and oil carriers for Acti-dione treatment of trunk canker wounds on reproduction and pole western white pine are described in this paper.

MATERIALS AND METHODS

Tests were made on two ages of western white pine in the Kaniksu National Forest: a 25- to 35-year-old natural stand in Nuisance Creek, and a 15-year-old plantation in Diamond Creek. Diseased trees in these stands were selected for treatment if the trunk was not endangered by the amount of bark girdled by the rust, and the foliage was of normal color and growth. Generally, trees contained what appeared as healthy bark on one-third or more of the trunk circumference. In the Nuisance Creek pole stand, rust infection was old. Trunk cankers were large and in pycniospore and aeciospore production. In contrast, trunk can-

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[1] Forester, Development and Improvement Unit, Division of Blister Rust Control, Region One, Forest Service, United States Department of Agriculture.

[2] Active ingredient 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide.

kers were small and in all structural stages of development on the younger trees in the Diamond Creek plantation.

The method used in these tests was as follows: Trees were banded by yellow paint marker; trunk cankers were numbered by aluminum tags. Limbs from the lower third of the crown height of trees were first pruned with a pair of shears or a saw to eliminate branch infections and the safety hazard in using a hatchet or knife to partially excise a trunk canker. Trunk cankers were removed by starting the cut 1 inch and 2 inches, reproduction and pole respectively, above the outer limit of surface discoloration and extending the cut downward along the visible canker margin to the proximal limit of surface discoloration. Basal trunk cankers were excised to ground level only. Then the cut surface of cankers was sprayed to the dripping point with Acti-dione stove oil solution using a quart-size pressurized fog dispersing Model A Sure Shot sprayer.[3] Slightly less than 3 quarts of solution were used in treating 68 trunk cankers on 60 pole-size trees varying from 6 to 10 inches in diameter breast height. Three pints of solution were used in treating 75 trunk cankers on 60 reproduction-size trees in the Diamond Creek plantation.

The chemotherapeutic solution was prepared by dissolving crystalline Acti-dione[4] at 150 ppmw in acetone and adding the solute to the aqueous and oil carriers. Acti-dione in each of three carriers was tested on 20 infected trees each of reproduction and pole western white pine. In the treatment of reproduction and pole check trees, trunk cankers were excised and the cut surface on five trees each sprayed to the dripping point with 1) water plus 2% glycerol, 2) Sovaspray 100, 3) No. 1 stove (fuel) oil, and 4) unsprayed. Trunk cankers were excised and treated in mid-August, 1956.

RESULTS AND DISCUSSION

Bark around trunk canker wounds was examined for mycelial activity in June and in August, 1957. The perseverance of rust in the trunk was determined by scrutinizing bark around wounds for 1) pycniospore and aeciospore production, 2) surface discoloration and swelling, 3) parasitism by the purple mold fungus, Tuberculina maxima Rostrup (2), and 4) callus tissue formation. Bark samples collected from questionable cankers in the field were sectioned and microscopically examined for blister rust hyphae in the laboratory. The results of these tests are presented in Table 1.

Number 1 stove oil proved particularly suitable as a carrier for Acti-dione. It is readily available at low cost and combines the following good features: high capacity for penetrating bark tissue, freedom from rapid loss by evaporation, satisfactory spreading properties, no objectionable smell or skin irritation, noncorrosiveness to sprayers, noncongelating at winter temperatures, and acute and chronic toxicants less concentrated than comparably priced diesel oil. Larvae and adult mountain pine beetles, Dendroctonus monticola Hopk., exposed by excising trunk cankers were killed by stove oil also.

The two other carriers used with Acti-dione proved unsatisfactory compared to No. 1 stove oil. Acti-dione in Sovaspray 100 was effective on blister rust mycelium in a lateral and downward direction but not in all cases upward from the margin of wounds. Acti-dione in aqueous glycerol solution was 100 percent effective below wounds only. Unlike Acti-dione Sovaspray 100, it often failed to kill blister rust mycelium laterally from a wound. The poor penetrating capacity of Sovaspray 100 and aqueous glycerol carriers for Acti-dione apparently results from the "pitching" faculty of wounds. Where wounds bled profusely, the trunk cankers usually survived treatment. Likewise, treatment was least effective in the lateral and distal portions of wounds. The results show that Acti-dione stove oil diffusion in bark tissue was not similarly affected by resin outflow. In this study, Acti-dione stove oil diffusion from cut edges was not separated from outer bark penetration probabilities.

None of the carriers showed phytotoxic or fungitoxic activity when applied to trunk canker wounds on check trees. Both the treated and untreated trunk cankers produced spore stages of the rust on check trees the next season, 1957.

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[3] Milwaukee Sprayer Manufacturing Company, Milwaukee, Wisconsin.

[4] The author gratefully acknowledges the cooperation of Dr. William Klomparens, Agricultural Research Division, The Upjohn Company, Kalamazoo, Michigan, in furnishing Acti-dione samples for these tests.

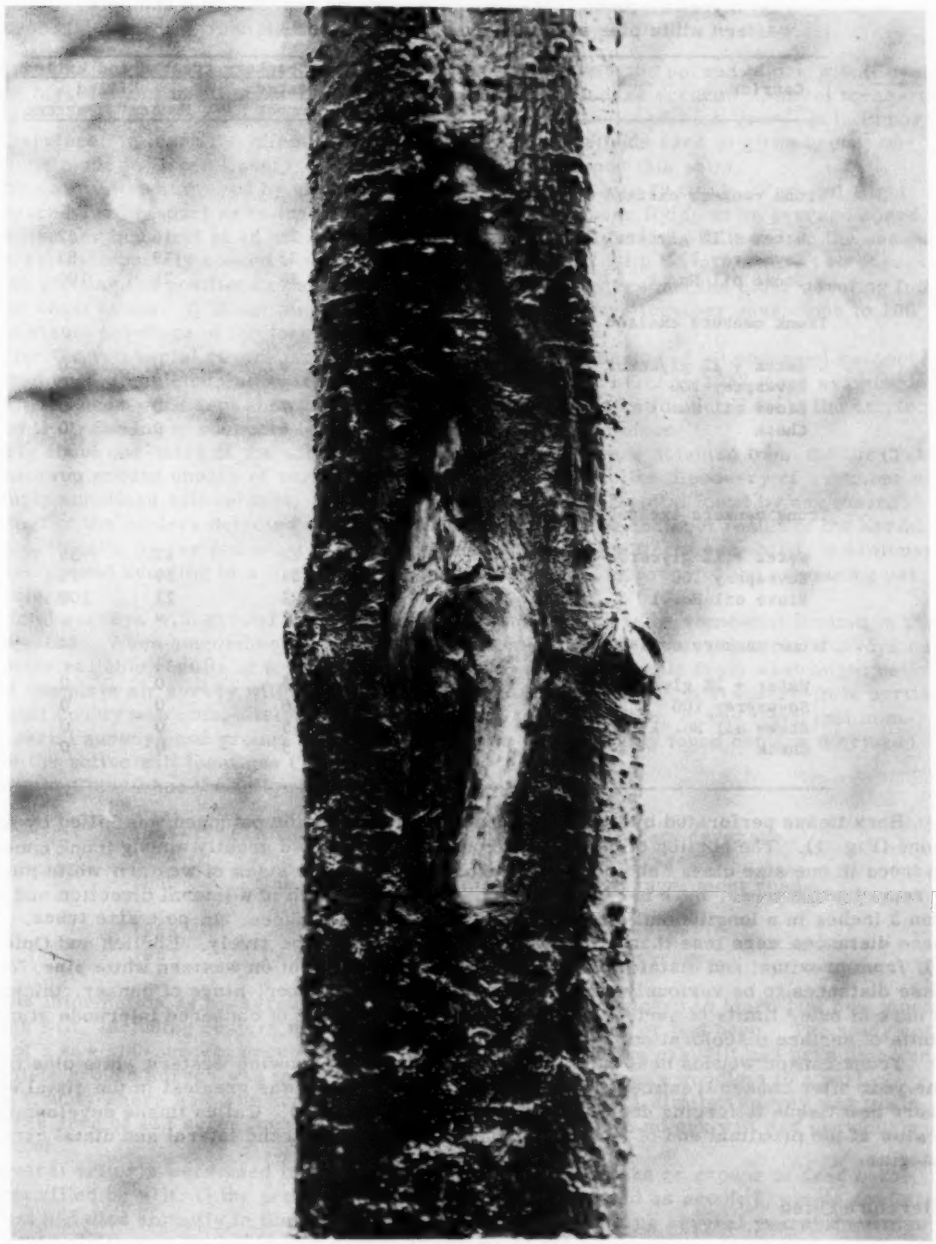

FIGURE 1. Pole-size western white pine showing Acti-dione treatment of blister rust trunk canker excised by starting cut 2 inches above outer limit of surface discoloration and extending cut downward along visible canker margin to proximal limit of surface discoloration. Bark tissue perforated by hyphae around wound died after pathogen was killed by Acti-dione. Callus tissue is forming 1 year following canker treatment in 1956.

Table 1. Effect of 150 ppmw Acti-dione in aqueous and oil carriers on blister rust mycelia around trunk canker wounds on reproduction and pole western white pine in the Kaniksu National Forest.

Carrier	Average age and number of treated trees		Trunk cankers treated and killed		
			Treated	Killed	
	Age	Number	Number	Number	Percent
REPRODUCTION					
Trunk cankers excised and Acti-dione treated					
Water + 2% glycerol	15	20	26	16	62
Sovaspray 100	15	20	23	19	83
Stove oil No. 1	15	20	26	26	100
Trunk cankers excised but not Acti-dione treated					
Water + 2% glycerol	15	5	6	0	0
Sovaspray 100	15	5	5	0	0
Stove oil No. 1	15	5	7	0	0
Check	15	5	6	0	0
POLE					
Trunk cankers excised and Acti-dione treated					
Water + 2% glycerol	30	20	22	12	55
Sovaspray 100	30	20	24	19	79
Stove oil No. 1	30	20	22	22	100
Trunk cankers excised but not Acti-dione treated					
Water + 2% glycerol	30	5	5	0	0
Sovaspray 100	30	5	6	0	0
Stove oil No. 1	30	5	5	0	0
Check	30	5	5	0	0

Bark tissue perforated by hyphae beyond cuts died after the pathogen was killed by Acti-dione (Fig. 1). The portion of dead bark around wounds varied greatly among trunk cankers on trees in one size class, and between reproduction and pole sizes of western white pine. In reproduction trees, bark necrosis extended less than 1 inch in a lateral direction and less than 3 inches in a longitudinal direction from cut edges of cankers. In pole size trees, these distances were less than 2 1/2 inches and 6 inches, respectively. Ehrlich and Opie (1), from proximal and distal measurements of mycelial extent on western white pine, found these distances to be variously correlated with the age of canker, stage of canker, thickness of bark at outer limits of surface discoloration, and diameter of cankered internode at outer limits of surface discoloration.

Trunk canker wounds heal quickly on young vigorously growing western white pine trees. One year after canker treatment in 1956, callus development was greatest in the distal end where new tissue is forcing dead bark from the wound (Fig. 1). Callus tissue development is slow at the proximal end of wounds compared with growth at the lateral and distal canker margins.

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DIVISION OF BLISTER RUST CONTROL, REGION ONE, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE.

OBSERVATIONS ON OAK WILT DETECTION IN TENNESSEE IN 1957

John S. Boyce, Jr., and W. A. Stegall, Jr.[1]

Attempts are being made by State forestry agencies to slow the spread of oak wilt (Ceratocystis fagacearum) in all of the eastern States where the disease occurs. Control measures are applied to as many actively wilting oaks as can possibly be found each year. It is important, therefore, to learn the effectiveness of the detection methods used in given areas; observations made in Green County, Tennessee, in 1957 bear upon this point.

The county was surveyed by air June 19 to 21, and again July 29 to 31. Parallel flight lines spaced 1 mile apart were traversed with a Piper Tri-Pacer flying at an average speed of 85 miles per hour and at an average height of 500 feet. Flight lines followed on the second survey were alternately spaced with those flown on the first. With the pilot were two observers, one plotting the positions of suspected active oak wilt centers and the other devoting full time for observation. It is estimated that the two aerial surveys together gave close to 100 percent visual coverage of the forests below.

After the two aerial coverages with subsequent ground checking of all observed suspect areas had been completed, all wilt centers that had been found prior to 1957 were examined on foot. Records were kept showing whether each center was first detected from the air, from a car while traveling to suspects located from the air, or from afoot.

Only about one-third of the active oak wilt centers found were detected from the air (Table 1). Followup ground checks of aerially detected suspects led to the discovery of a number of previously unnoticed wilt centers, amounting to 18 percent of the total found by any means. About half of the centers detected were thus found as a direct or indirect result of the aerial survey. Revisits by car and afoot to known centers added a significant one-third. Additional intensive ground scouting in a limited area accounted for the discovery of the remaining oak wilt centers.

Aerial surveys with ground checks were thus demonstrated to be somewhat limited in their effectiveness. When supplemented by revisits to known centers, however, aerial surveys can yield more reliable results at a reasonable cost. To compare results from what might be termed complete air survey with those from thorough ground survey, a 4-square-mile portion of the test county was completely ground scouted. In this area, too, it was found that combining aerial surveys and ground followups with visits to previously found centers disclosed most of the active wilt locations (Table 1).

Greene County has the following oak wilt survey history:

1951-1953	Roadside and aerial surveys; 10 percent coverage.
1954	One aerial survey; 100-percent coverage.
1955	One aerial survey; 60-percent coverage; most roadsides scouted.
1956	One aerial survey; 100-percent coverage.
1957	Two aerial surveys; 100-percent coverage.

With this information as background material, a study was undertaken to learn just how much total oak wilt, including centers now inactive, there actually was or had been. The study consisted of a new 100-percent ground survey in an area where active oak wilt had been detected. A complete ground survey was made of 789 acres composed of many woodlots in a part of Greene County where wilt was known to be relatively abundant. A similar survey was made in a check area of 423 acres located in a part of the county where no oak wilt had previously been found.

Several criteria were used in judging whether single dead oaks or groups of dead oaks had been killed by wilt: 1) the presence of mat scars was accepted as proof; 2) single dominant oaks that had died abruptly in the spring or early summer following several years of normal growth were tallied as probable wilt cases; 3) groups of oaks containing stems that had died at different times but which showed an arrested final growth ring were also tallied as probable wilt centers.

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Table 1. Oak wilt survey results in Greene County, Tennessee, 1957.

Area	Total centers known active in 1957	Oak wilt centers found from-- Air	Air plus followup	Air plus followup, plus revisit old centers	Air, followup, revisit, other ground scouting
	Number			Percent	
Entire county (580 sq. mi.)	49	35	53	86	100
Area 100-percent ground surveyed (4 sq. mi.)	19	42	53	84	100

Table 2. Results of oak mortality survey in parts of Greene County, Tennessee, 1957.

	Groups of dead oaks: With mat scars	Groups of dead oaks: Probable wilt[a]	Single dead oaks: With mat scars	Single dead oaks: Probable wilt[b]	Confirmed wilt through 1957: Groups	Confirmed wilt through 1957: Single trees
	Number					
Wilt area, 789 acres	2	24	3	44	15	3
Check area, 423 acres	0	1	0	4	0	0

[a]Progressive dying, arrested growth, no mat scars.
[b]Dominant trees only, arrested growth, no mat scars.

With regard to the third criterion, diagnosis on the basis of a prematurely arrested final growth ring is supported by observations that oaks which die from wilt infections characteristically exhibit such a growth pattern. This general observation was specifically checked in the spring of 1957, when the radial growth of 21 wilt-killed trees that had shown symptoms in 1956 and of 20 healthy nearby oaks of the same diameter classes was measured. Radial growth for 1956 was expressed as a percentage of the 1951-1955 radial growth average. This percentage was found to be 32 for the wilt-killed oaks and 105 for the healthy trees.

The intensive ground survey in the 789-acre area turned up five previously undetected inactive centers that had mat scars on at least one tree at each spot in addition to the 18 confirmed centers that had been located during 1951-1957 (Table 2). An additional 24 groups of trees very probably wilt killed were recorded, as well as 44 single, dominant, dead oaks each with an arrested final growth ring. These data, indicating a high incidence of previously undetected wilt in in this area, are particularly significant when compared with the data on the 423-acre check area, on which no dead oaks with mat scars and only one likely wilt group and four single dominant oaks with arrested growth were found. The results reported herein, interpreting the cause of oak mortality in the wilt area of Greene County, Tennessee, support the findings of Gillespie and Craig in West Virginia[2].

Only 20 percent of the total probable centers in the wilt area were detected by the surveys, which were conducted over a period of several years. This figure has greater qualitative than quantitative significance, however, because some of the centers may never have been active during the period of survey activities. Nevertheless, even when we allow for the possibility that some oaks were incorrectly judged to have died of oak wilt, it is evident that there has been much more wilt in this area than the previous surveys have disclosed.

[2] Gillespie, W. H., and F. W. Craig. 1958. An attempt to evaluate the significance of dead oak trees found in oak wilt sites in West Virginia. Plant Dis. Reptr. 42: 268-271.

There are several possible reasons why the surveys in Greene County did not detect more of the wilt over a period of several years. First, it is likely that wilt trees with good symptoms have simply been overlooked by the aerial observers. Second, it is even more likely that infected trees have not been detected because symptoms were poor or lacking at the time of the surveys. With reference to this probable weakness, several moribund oaks with poorly defined symptoms were culturally sampled in May 1957 and proved to be wilt cases. The main reason for attempting isolations from them was their proximity to known wilt trees. The same centers had been examined during the summer of 1956, and the trees did not show wilt symptoms at that time. A third reason why the surveys failed to pick up all infections is that many centers do not have newly wilting oaks each succeeding year, but rather, there may be intervals of one or two years between the appearance of symptom trees.

It is thus likely that one or two aerial surveys per year of a wilt area, starting after June 15, even with essentially complete coverage and even when repeated for as many as 3 years in succession, will enable us to find only a minority of the standing infected trees. By starting early enough and repeatedly surveying the same area during the same season, however, we can undoubtedly detect most of the symptom trees.

The evaluation of the results of oak wilt detection and control efforts should take into account the fact that some trees not showing marked symptoms will probably remain undiscovered, and that there are limitations inherent in attempting to locate all infected trees, as illustrated by the Greene County experience.

UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST SERVICE,
SOUTHEASTERN FOREST EXPERIMENT STATION, ASHEVILLE, NORTH
CAROLINA

OAK WILT DISTRIBUTION THROUGH 1957

T. W. Bretz and T. W. Jones[1]

The general area of oak wilt distribution in the United States was fairly well defined in 1951[2]. Since then, the disease, which is caused by the fungus Ceratocystis fagacearum (Bretz) Hunt, has been reported from many additional locations within this general area. Hepting[3] reported its distribution in this country through 1954.

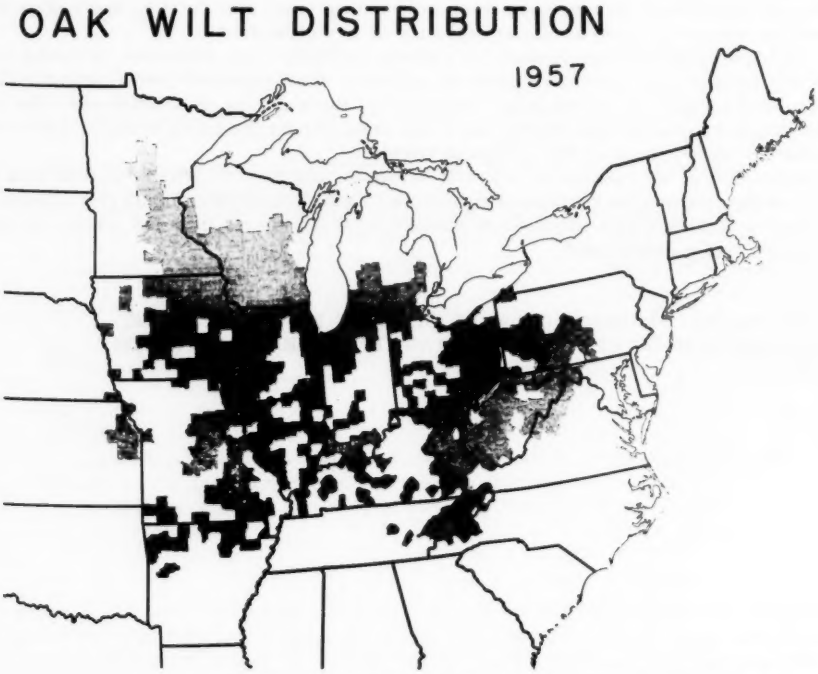

FIGURE 1. Known distribution of oak wilt in the United States through 1957, by counties.

The accompanying map (Fig. 1) shows the known distribution of oak wilt through 1957, by counties. The data upon which the map is based were supplied by oak wilt investigators working in the various States. All counties in which at least one case of the disease has at some time been reported are included. It is not known if oak wilt was present in all of them in 1957.

MISSOURI AGRICULTURAL EXPERIMENT STATION IN COOPERATION WITH
THE CENTRAL STATES FOREST EXPERIMENT STATION, FOREST SERVICE,
UNITED STATES DEPARTMENT OF AGRICULTURE

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[1] Respectively, Professor of Forestry, School of Forestry, University of Missouri, Columbia, Missouri, and Pathologist, Central States Forest Experiment Station, Forest Service, United States Department of Agriculture, Columbia, Missouri. Contribution from the Central States Forest Experiment Station, Forest Service, United States Department of Agriculture, and Missouri Agricultural Experiment Station, Journal Series No. 1862. Approved by Director.

[2] Fowler, Marvin E. 1952. Oak wilt surveys in 1951. Plant Dis. Reptr. 36: 162-165.

[3] Hepting, George H. 1955. The current status of oak wilt in the United States. Forest Sci. 1: 95-103.

DISEASES OF WOODY HOSTS NEW TO ILLINOIS

Dan Neely[1]

From the leaf and stem specimens of woody hosts mailed to this laboratory for diagnosis and from collections made on field trips by G. H. Boewe and R. A. Evers, members of the Natural History Survey staff, several fungi and one bacterium have been found on hosts on which they have not been previously reported in Illinois. These pathogens are listed in Table 1. After referring to the indices prepared by Saccardo (4), Seymour (5) and Weiss (6) it is believed that five of the fungi have never before been found on these hosts in the United States (Table 1).

Table 1. Pathogens found on woody hosts for the first time in Illinois.

Pathogen	Host	Collected	
		Place	Date
Cercospora pteleae Winter	Ptelea trifoliata L.	Oregon	Sept. 9, 1957
Cercospora ribis Earle	Ribes grossularia L.[a]	Champaign	Aug. 8, 1957
Dothiorella sp.	Acer saccharum Marsh[a]	Morton	Jan. 12, 1958
Dothiorella sp.	Populus simonii Carr.[a]	Champaign	Oct. 10, 1957
Entomosporium thuemenii (Cke.) Sacc.	Crataegus monogyna Jacq.	Fairbury	Oct. 15, 1938
Gloeosporium fagi (Desm. & Rob.) West.	Fagus grandifolia Ehrh.	Mounds	May 22, 1947
Microsphaera alni DC. ex. Wint.	Euonymus europaeus L.[a]	Urbana	Oct. 4, 1957
Oidium sp.	Rhus aromatica Ait.	Manhattan	Aug. 3, 1957
Pseudopeziza ribis Kleb.	Ribes alpinum L.	Chicago	June 11, 1957
Puccinia caricis (Schum.) Schroet. var. grossulariata Arth.	Ribes cynosbati L.	Caledonia	May 26, 1954
Tubercularia sp.	Euonymus alatus (Thunb.) Sieb.[a]	Spring Valley	Oct. 21, 1957
Agrobacterium tumefaciens (E. F. Sm. & Town.) Conn	Euonymus fortunei (Turcz.) Hand.-Maz.	Glencoe	Mar. 1, 1958
Agrobacterium tumefaciens (E. F. Sm. & Town.) Conn	Euonymus japonicus L.	Urbana	Mar. 5, 1957

[a] New hosts for the United States.

Of the five fungi on hosts new to them in the United States, Dothiorella spp. and Tubercularia sp. inhabit bark and Cercospora ribis Earle and Microsphaera alni DC. ex. Wint. inhabit leaves. Dothiorella sp. on sugar maple appeared to be causing dieback of twigs on trees injured by periodical cicada. The fungus was fruiting only on twigs injured by the insect. Sporulation of Dothiorella sp. on Simon poplar was obtained only after cankered sections of large branches were placed in moist chambers for 14 days. The species of Dothiorella from each of these hosts was similar to Dothiorella quercina (Cke. & Ell.) Sacc. (1). Tubercularia sp. on winged euonymus was fruiting profusely on the large and intermediate branches which were dead at the time of collection. The dotting of the bark by black, erumpent, pulvinate sporodochia completely encircled the stems. This fungus appears identical to Tubercularia ulmea Carter (2). The Cercospora leaf spots on Ribes grossularia L. were irregular to circular in shape and measured from 1 to 4 millimeters in diameter. The spots were composed of dark purplish-brown margins surrounding a dingy gray center. Amphigenous fruiting of the fungus was readily observed. The fungus is identical with Cercospora ribis Earle, which is reported on three other species of Ribes by Chupp (3). Leaves from European spindle-tree, Euonymus europaeus, collected in the fall had their lower surfaces covered with the powdery mildew, Microsphaera alni DC. ex. Wint. Weiss (6) has reported Microsphaera alni on three other species of Euonymus.

[1] Assistant Plant Pathologist, Illinois State Natural History Survey, Urbana, Illinois.

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ILLINOIS STATE NATURAL HISTORY SURVEY, DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, URBANA, ILLINOIS

BRIEF NOTES

HAWTHORN SPRAYING IN PENNSYLVANIA WITH ZINEB +

By L. P. Nichols[1]

Davis (1) reported severe injury to an unnamed species of hawthorn following spray applications of zineb at the Morris Arboretum in Philadelphia in 1945. In 1953 in New Jersey, Bachelder and Dochinger (2) found slight injury to the foliage of English hawthorn, Crataegus oxycantha, following one, two, three and four applications of zineb, 1 1/2-100. In 1955 trees of Paul's scarlet hawthorn, C. oxycantha pauli, on the campus of the Pennsylvania State University were sprayed with zineb for the control of the leaf blight caused by Fabraea maculata. Adjacent trees of the Washington hawthorn, C. phaenopyrum were sprayed with the same material at the same time. A power hydraulic sprayer of the estate type was used. It was later reported that although good control of the leaf blight on the Paul's scarlet hawthorn was obtained, severe injury and defoliation occurred on the Washington hawthorn.

In 1956 trees of cockspur thorn C. crus-galli, Washington hawthorn, C. phaenopyrum, C. arnoldiana, C. lavalli, C. arkansana, C. mollis, Paul's scarlet hawthorn, C. oxycantha pauli, English hawthorn C. oxycantha and an unknown variety from Lebanon were sprayed with two formulations of zineb (Parzate Zineb Fungicide and Dithane Z-78) at the rate of two pounds per 100 gallons of spray. Three gallon compressed air sprayers were used. The cockspur thorn and Washington hawthorns were sprayed on June 22, June 26, July 7 and July 14. The other varieties were sprayed on July 7 and 14. On each tree two branches were sprayed with Parzate and two branches were sprayed with Dithane Z-78. A check on July 20 and several times later showed no evidence of injury on any variety. On September 1 a check of the Paul's scarlet hawthorn tree showed that branches sprayed with zineb had only traces of leaf blight while the unsprayed portions of the trees were almost completely defoliated.

In 1957 the same sprays were applied to the same trees. All varieties were sprayed on April 27, May 11, May 25, and June 10. No injury was observed on any variety in 1957. Once again branches of Paul's scarlet hawthorn sprayed with zineb showed only traces of leaf blight while in spite of the extremely dry summer the unsprayed branches were almost completely defoliated.

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POOR GROWTH OF ROSES IN COMMERCIAL GREENHOUSE BEDS INFESTED WITH THE FUNGUS, LEPIOTA MORGANI +

By A. F. Schindler[1]

During the winter of 1956-57 several commercial rose growing greenhouses were found to be or reported as having beds or parts of beds where roses were growing poorly. Upon examination the soil was found to be heavily infested with the mycelium of a fungus. In most instances fruiting bodies were abundant. Several were identified by John A. Stevenson, of the Mycology and Plant Disease Reporting Section, Agricultural Research Service, as Lepiota morgani Pk. and placed in the National Fungus Collections, Beltsville, Maryland.

This condition in roses appears to be similar to the effects produced in grasses and other herbaceous plants by this and other related Basidiomycetous fungi which often grow in "fairy rings". Shantz and Piemeisel (3), in their study of fungus fairy rings, state that "the development of the mycelium brings about chemical and physical changes in the soil", and that the death of the vegetation is due to a number of different causes. Ramsbottom (2) concluded that the detrimental effects were due to a "physiological drought". Some of the rose growers observed the inability of water to percolate down through the soil in those areas where the fungus mycelium was present in appreciable amounts.

The mushroom spawn nematode (Ditylenchus sp.) is known to feed on the mycelium of the commercial mushroom, Agaricus campestris Fr., and when inoculated on a large number of seed plants it does not multiply or cause any detrimental effects (1). Therefore, it was felt that possibly this nematode might also feed on the mycelium of L. morgani while not harming the rose plants.

In addition to several sections of a commercial greenhouse bed which were infested with the fungus, inoculations of Ditylenchus were made into cultures of L. morgani in the laboratory. One of these laboratory cultures produced a fruiting body which was also identified as L. morgani. At the same time inoculations were also made into cultures of A. campestris. The nematodes survived and multiplied on the A. campestris cultures but failed to survive on the cultures of L. morgani. It was therefore concluded that biological control of L. morgani with mushroom spawn nematode was not feasible.

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ANNOUNCEMENT

The 37th Annual Report of the Canadian Plant Disease Survey (1957) will soon be ready for distribution. Some copies of the 36th Report (1956) are still available. Requests from pathologists interested in receiving either of these Reports will be considered until supplies are exhausted. Such requests should be directed to:

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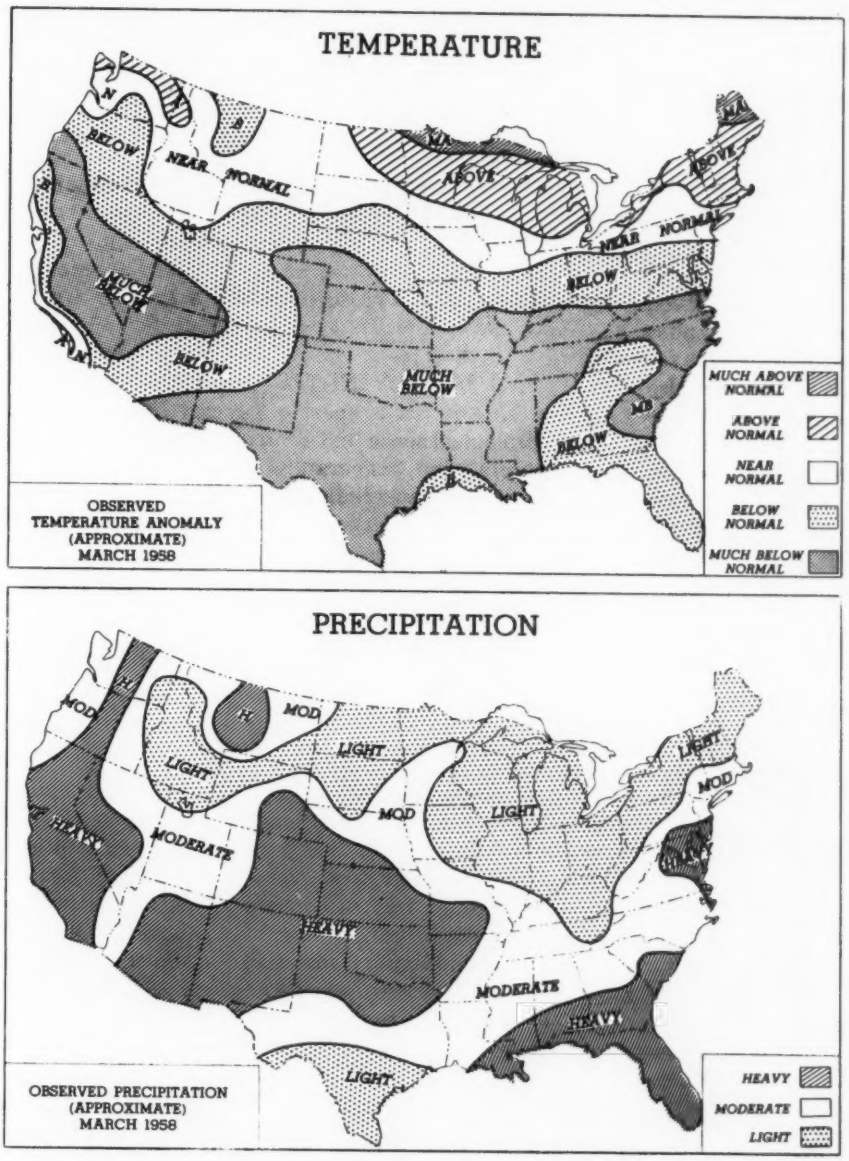
Botany and Plant Pathology Division
Canada Department of Agriculture
Science Service Building
Carling Avenue
Ottawa, Ontario



The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.